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Nafarelin Acetate for Pituitary Suppressions in In-Vitro Fertilisation Cycles – A Singaporean Experience

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ABSTRACT

Aim of Study: The aim of this prospective clinical trial was to determine if intranasal nafarelin acetate (NA) is as effective as leuprolide (LA), our standard GnRHa, in IVF cycles. In addition, we believe that this may be the first report of such a trial in an Asian IVF population.

Method: Midluteal GnRHa administration (LA = 0.5 mg/d; NA = 800 μg/d) was used with a standardised hMG ovarian stimulation protocol for all 88 consecutive cycles, randomised at recruitment.

Results: There were no significant differences between LA and NA in terms of the mean duration of agonist to reach pituitary suppression, total hMG dosage, number of embryos produced or frozen and the clinical pregnancy rate (LA = 21.4% and NA = 16.3% per cycle).

Conclusion: Intranasal nafarelin acetate was as effective as leuprolide acetate in our series of IVF patients of Asian origin, and may be offered as an alternative choice for pituitary suppression.

Keywords: nafarelin acetate, pituitary suppression, in vitro fertilisation

INTRODUCTION

Nowadays, most IVF centres in the world use some form of gonadotrophin-releasing hormone agonist (GnRHa) before ovarian stimulation in IVF cycles. The use of GnRHa reduces the circulating levels of bioactive luteinising hormone (LH)[1], thus preventing premature LH release and undesirable effects of excessive luteinisation on oocyte quality[2]. Lower levels of LH also improve local hormonal millieu in the follicle, thus improving the quality of the oocytes[3]. The average number of oocytes obtained at retrieval are also markedly increased[4,5].

Several GnRHa have been used clinically by IVF units but most of them are administered as injectables. Since 1994, our IVF centre has been using leuprolide acetate (Lucrin®), a short-acting once-daily subcutaneous injection of GnRHa, for midluteal pituitary suppression (the long protocol). However, these injections are inconvenient especially when the gonadotrophin injections are started, as the two medications cannot be administered in the same syringe. Also, a small proportion of patients are uncomfortable about administering the injections themselves. There are also the added costs of needles and syringes and the necessity of proper disposal of these "sharps".

Nafarelin acetate (Synarel®) was first introduced in Singapore for the treatment of endometriosis. It is administered in a painless and relatively convenient way, ie. as an intranasal spray. We conducted a small prospective randomised study to determine if nafarelin acetate could be an effective non-injectable GnRHa for pituitary suppression in our local IVF population. We compared this with the "standard" GnRHa used in our centre, leuprolide acetate, using the long protocol method.

METHODS

Eighty-eight consecutive first IVF cycles patients were recruited into this study over an 8-month period and were randomised into the two arms of the study. Patients who had contraindications to either nafarelin or leuprolide were excluded from this prospective study. Those who declined to participate in the study, and those who had been scheduled for a gamete intrafallopian transfer procedure were also excluded. There were 42 patients in the leuprolide group (group I) and 46 in the nafarelin group (group II). All patients were taught by our four nurses the correct method of drug administration.

GnRHa was started from the midluteal phase of the cycle. Leuprolide was self-administered by daily subcutaneous injections as 0.1 mL (0.5 mg/day) doses. Nafarelin was administered as a twice-daily nasal spray with 2 puffs given each time (total of 800 μg/day). The dosages of GnRHa were kept constant throughout the treatment cycle. Adequate pituitary suppression was determined by serum estradiol (E_2) levels and ultrasonographic evaluation of the ovaries and endometrium. This assessment begun after a minimum of 10 days of GnRHa treatment. Treatment was continued and repeat assessments were performed at 5 to 7-day intervals until the downregulation criteria was reached ie. serum estradiol level of $\leq$ 50pg/mL

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and all ovarian follicles < 10 mm in size with absence of any functional ovarian cysts. If a patient failed to reach these levels of pituitary suppression after 4 weeks of GnRHa, treatment was deemed to have "failed" and they were then either offered cancellation of the cycle or conversion to the alternative choice of GnRHa (ie. from Synarel to Lucrin or vice-versa). "Converted" cycles were thereafter excluded from the analysis and final outcome.

The stimulation regime was identical in both groups. We used daily intramuscular injections of human menopausal gonadotrophin (hMG) during the stimulation phase. The daily dosage and duration of hMG therapy was individualised according to the patient's ovarian response. Monitoring was via serial ultrasonographic assessments by 2 experienced registrars. When at least 3 dominant follicles greater than 18 mm size were present, a standard ovulating dose of 10,000 IU human chorionic gonadotrophin (hCG) was given and transvaginal ultrasound-guided oocyte retrieval was scheduled for 34 to 38 hours after this. Decisions regarding the stimulation doses and responses were made independent of the particular agonist used. GnRHa therapy was discontinued once the hCG injection was given.

Oocyte retrievals were performed by two experienced specialists in an outpatient setting using mainly light sedation and analgesia with fentanyl and midazolam. Semen was collected on the morning of the retrieval and prepared for routine dish insemination 4 to 6 hours later. Fertilisation was assessed 16 to 20 hours after insemination. Cleavage and embryo grading were performed at 46 to 48 hours after retrieval. Transcervical intrauterine transfers were performed 2 days after retrieval, with a maximum of 3 embryos being transferred. Extra embryos of adequate morphological grade were cryopreserved for future transfers (according to our unit protocol for embryo selection for freezing). Luteal phase support was again standardised with daily intramuscular Progesterone 50 mg injections for the next 17 days.

Serum for βhCG level was taken 19 days after oocyte retrieval and clinical pregnancies were confirmed by the presence of a gestational sac on ultrasound assessment 4 to 5 weeks later. Only clinical pregnancies were included in this analysis. Ectopic pregnancies were considered "clinical" pregnancies for the purpose of this report.

Outcome assessments included cancellation rate (prior to starting hMG), duration to achieve pituitary suppression, serum estradiol level after 10 days of GnRHa, total dose of hMG required, number of oocytes retrieved, fertilisation and cleavage rates, embryo transfer rate, number of embryos cryopreserved, clinical pregnancies and livebirth rate per retrieval. Patients on nasal spray were also questioned on the occurrence of side-effects possibly linked with this route of administration eg., sneezing, rhinitis and pain or inflammation of nasal mucosa.

Statistical comparison of means was made with two-tailed Student's t-test. Outcome data was analysed with Fisher's exact test. The calculations were made using Microsoft Excel Tools and Graphpad Instat Software packages. Results were considered statistically significant when the p-value was less than 0.05.

Table I – Patient characteristics by GnRHa

Variable	Leuprolide	Nafarelin	p value*
Age (yrs) – mean+	34.8 ± 0.7	34.6 ± 0.6	NS
– range	24 to 43	24 to 41	
Major etiology (% of patients)			
– ovulatory disorders	26.2%	30.4%	NS
– tubal disease	21.4%	41.3%	NS
– male infertility	66.7%	63.0%	NS
– endometriosis	23.8%	17.3%	NS
– unexplained	0.0%	2.2%	NS
% patients with combined male & female factors	28.6%	43.5%	NS

* NS = not significant
significance assumed at $p < 0.05$
\+ mean ± SEM

Table II – Pituitary response by GnRHa type

Variable	Leuprolide	Nafarelin	p value*
No. of cycles	42	46	
Failed suppression	0/42 (0.0%)	3/46 (6.5%)	NS
Cysts present	0/42 (0.0%)	4/46 (8.7%)	NS
Mean d10 serum Estradiol (pg/mL)+	107.4 ± 18.5	162.4 ± 47.3	NS
Mean days of GnRHa to reach suppression+	14.0 ± 0.8	15.2 ± 0.9	NS

* NS = not significant
significance assumed at $p < 0.05$
\+ mean ± SEM

RESULTS

Of the 88 consecutive IVF cycles, 48% (42) used leuprolide whilst 52% (46) used nafarelin. Patient characteristics (Table I) between the two groups were not significantly different with respect to age and etiology of their infertility. Once recruited into the study, no patients dropped-out of the study.

Table II shows that the response to GnRHa therapy with respect to pituitary suppression was also not significantly different between the two groups. However, it is important to note that a larger proportion of patients (21.7%) on nafarelin needed prolonged treatment, 3 weeks or more (Table III), to reach adequate pituitary suppression. In addition, 3 patients using nafarelin failed to be suppressed despite more than 28 days of treatment. Two of these 3 patients were converted to leuprolide and subsequently successfully suppressed after 10 days of conversion.

We also looked at whether using a different GnRHa had any effect on ovarian stimulation. The nafarelin group required overall fewer ampoules of hMG (not statistically significant) and produced more oocytes at retrieval; mean of 10.93 oocytes versus 7.95 for the leuprolide group. Although this difference was statistically significant, it did not seem to result in more embryos being produced or frozen. It was also noted that there were no cycles cancelled during

Table III – Duration of GnRHa usage to achieve pituitary suppression

GnRHa usage (days)		Leuprolide	Nafarelin
10	"satisfactory"* response	54.8% (23)	37.0% (17)
11 – 15		9.5% (4)	17.4% (8)
16 – 20		21.4% (9)	23.9% (11)
21 – 30	"poor" response	14.3% (6)	13.0% (6)
⩾ 30		0.0% (0)	8.7% (4)
Overall range		10 to 30	10 – 38

* empirically taken as "satisfactory" response

(p value was not significant when comparing proportion of cycles with satisfactory response between the two groups)

Table IV – Ovarian stimulation phase by GnRHa

Variable	Leuprolide	Nafarelin	p value*
Total hMG used (amps)#	46.3 ± 2.95	41.7 ± 2.45	NS
Total oocytes retrieved#	7.95 ± 0.72	10.93 ± 1.15	SIG+
% oocytes fertilised	205/334 = 61.4%	277/467 = 59.3%	NS
% embryos cleaved	199/205 = 97.1%	262/277 = 94.6%	NS
No. of 2PN embryos/retrieval#	192/42 = 4.6 ± 0.7	236/42 = 5.5 ± 0.9	NS
No. of frozen embryos/retrieval#	98/42 = 2.3 ± 0.5	126/42 = 3.0 ± 0.8	NS
% cycles reached ET	31/42 = 73.8%	33/44 = 75.0%	NS
% cycles with frozen embryos	20/31 = 64.5%	18/33 = 54.5%	NS

* NS = not significant; SIG = statistically significant

\# data presented as mean ± SEM

\+ p value = 0.03

ovarian stimulation for the leuprolide group and only one cancelled cycle for the nafarelin group.

Although our primary objective was to compare nafarelin with leuprolide with regards to pituitary suppression and ovarian stimulation characteristics, we also followed-up on these treatment cycles to assess their final pregnancy outcomes. The clinical pregnancy rate per cycle was 21.4% (9/42) for leuprolide group and 16.3% for the nafarelin group (not statistically significant). The livebirth rate per cycle was also similar between the two groups (14.3% and 16.3% for leuprolide and nafarelin respectively).

Finally, as a whole, the use of a nasal spray for the nafarelin group was well-accepted. Only a few patients had mild rhinitis and none had pain, inflammation of nasal mucosa or sneezing.

DISCUSSION

The advantages of IVF cycles pre-treated with GnRHa agonists have been well-documented. The composition of the synthetic decapeptide determines its potency relative to the native hormone. Leuprolide acetate has approximately 15 times the potency of gonadotrophin-releasing hormone and can be administered subcutaneously on a daily basis. Nafarelin acetate is 200 times more potent than native gonadotrophin-releasing hormone and can be administered intranasally[6]. The intranasal route is of course, the less invasive method of daily administration and also the more convenient one.

We compared these 2 agonists, administered via different routes, using midluteal pituitary suppression prior to a similar ovarian stimulation protocol. The doses used are those commonly employed in clinical practice for IVF cycles. In a previous clinical study involving 160 women on this method of pituitary suppression, 86% of those receiving 200 μg BD and 100% of those receiving 400 μg BD nafarelin achieved adequate downregulation. As the 200 μg BD dose did not produce ideal downregulation in all the women in this pivotal trial[6], we decided to allocate all our women the 400 μg BD dosage of nafarelin.

The patients were randomised to reduce any selection biasness in the study. On the whole, the group receiving nafarelin performed as well as the group using our "standard" GnRHa, leuprolide. Although the following results did not reach statistical significance, we feel that it is important to note that a fair proportion of patients using nafarelin took more than 3 weeks to achieve pituitary suppression. We feel that our patients should take note of this consideration when choosing nafarelin over leuprolide for their IVF cycle. In fact, 6.5% failed to reach suppression despite more than 4 weeks of treatment.

Like other researchers[7,8], we also noticed a reduction in the hMG requirements for the nafarelin group, though this was not shown statistically in our small study. There was a trend toward recovering a greater number of oocytes in the nafarelin group. However, this did not translate into any significant increase in embryos produced or surplus quality embryos for freezing, unlike an earlier report from Yale[7]. In addition, Martin et al[9] in their retrospective analysis of 510 IVF cycles, found that the nafarelin group had a higher number of delivered pregnancies per retrieval. We did not notice an increase in clinical pregnancy rate or livebirth rate for the nafarelin group. Several other researchers also came to a similar finding as ours[7,8,10,11].

In conclusion, the results of our present series showed that the use of intranasal nafarelin acetate was associated with a low incidence of cycle cancellation and a relatively good ovarian response in patients of Asian ethnicity. As both the GnRHa seem to be of almost identical efficacy, nafarelin acetate may thus be offered as an alternative choice for our patients, with the understanding that the convenience of a non-injectable may sometimes be off-set by longer duration of therapy.

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