A comparison of three gonadotrophin-releasing hormone analogues in an in-vitro fertilization programme: a prospective randomized study

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The use of gonadotrophin-releasing hormone analogues (GnRHa) has resulted in improved pregnancy rates in in-vitro fertilization (IVF) treatment cycles. Traditionally, short-acting analogues have been employed because of concerns over long-acting depot preparations causing profound suppression and luteal phase defects adversely affecting pregnancy and miscarriage rates. We randomized 60 IVF patients to receive a short-acting GnRHa, nafarelin or buserelin, or to receive a depot formulation, leuprorelin, all commenced in the early follicular phase and compared their effects on hormonal suppression and clinical outcome. We found that on day 15 of administration there was a significant difference in the suppression of oestradiol from initial concentrations, when patients on buserelin were compared with patients on nafarelin or leuprorelin (54 versus 72 and 65%; \( P < 0.05 \)) and also in the number of patients satisfactorily suppressed, (80 versus 90 and 90%; \( P < 0.05 \)), though there were no differences between the analogues by day 21. Similarly there was no difference in hormonal suppression during the stimulation phase or in implantation, pregnancy or miscarriage rates in comparing the three agonists. We conclude that with nafarelin and leuprorelin, stimulation with gonadotrophins may begin after 2 weeks of suppression and that long-acting GnRHa are as effective as short-acting analogues with no detrimental effects on the luteal phase.

Key words: GnRHa/hormonal suppression/IVF/pregnancy

Introduction

Gonadotrophin-releasing hormone agonists (GnRHa) are modifications of the parent decapeptide, GnRH, and their action is based upon reversible blockade of pituitary gonadotrophin release, resulting in suppression of ovarian function. Over the last decade, almost all in-vitro fertilization (IVF) centres have employed GnRHa for pituitary suppression before human menopausal gonadotrophin (HMG) administration. This has been shown to result in superior pregnancy rates when compared with cycles not utilizing analogues (Meldrum et al., 1983; Meldrum et al., 1984; Smitz et al., 1992; Testart et al., 1993), benefits ‘poor responders’ (Lounaye et al., 1988; Belaisch-Allart et al., 1988; Ben-Rafael et al., 1990) and reduces the number of cancelled cycles (Kerin, 1989; Lounaye, 1990; Hughes et al., 1992). Finally, with analogue use, better timing of the cycle is realized (Juha et al., 1993), allowing for improved patient and clinician convenience (Porcu et al., 1995). Most reports comparing the use of analogues in the long protocol have analysed cycles with the analogues being started in the mid-luteal phase.

The aim of this study was to compare three GnRH analogues: buserelin as a s.c. injection, nafarelin as an intranasal spray, both used routinely in this unit, and leuprorelin, as a long-acting depot preparation, all commenced in the early follicular phase in a long protocol regime. We particularly wanted to compare any evidence of escape from suppression between the long-acting depot with the shorter-acting analogues and also to investigate whether leuprorelin would have any detrimental effects in the luteal phase, because of its longer duration of action.

Materials and methods

Sixty patients undergoing IVF–embryo transfer treatment at the Assisted Conception Unit, St James’s University Hospital, Leeds, were evaluated for this study. Patients were required to be <38 years old, have had no more than two previous IVF attempts, a normal semen analysis as defined by World Health Organization (WHO, 1987) criteria, no ovarian surgery, no baseline ovarian cysts >20 mm diameter, a normal menstrual history and no major systemic disease or uterine abnormality. Recruited patients were randomized into three groups, receiving buserelin, nafarelin or leuprorelin for pituitary suppression.

GnRHa regime

Group A patients \( (n = 20) \), received s.c. buserelin acetate (Hoechst, Hounslow, Middlesex, UK), at a dose of 0.5 mg daily. Group B patients \( (n = 20) \), received nafarelin intranasal spray (Searle, High Wycombe, UK), 100 µg 8-hourly. Both drugs were started on the first day of the menstrual period (day 1), and continued throughout the cycle until the day human chorionic gonadotrophin (HCG) was administered. Patients in group C \( (n = 20) \) received a single s.c. depot injection of leuprorelin acetate (Prostap SR; Searle, Lederle Laboratories, Gosport, Hants, UK) 3.75 mg on day 1. Ovarian stimulation in all groups commenced after 21 days of pituitary desensitization. Patients were considered desensitized when an ultrasound scan confirmed an endometrial thickness <3 mm

Clinical protocol

Multiple follicular stimulation was achieved with highly purified urofollitropin Metrodin HP (Serono, Welwyn Garden City, Herts, UK).
In our unit the regimen for gonadotrophin (Metrodin) administration is a sliding scale based on the age of the patient. Women ≤34 years of age receive 3 ampoules (225 IU) daily; 35-38 years, 4 ampoules (300 IU) daily; and >38 years, 6 ampoules (450 IU) daily. Tracking of follicular growth was performed using transvaginal ultrasonography (Kretz, Technik, Combi 410, Tiefenbach, Austria) on day 6 of gonadotrophin stimulation and again on day 8. The mean maximal follicular diameter was calculated from four measurements of the leading follicle, in two planes at 90° to each other. When two or more follicles had a mean diameter of 16-20 mm, HCG (Pregnyl; Organon, Cambridge, UK) was administered at a dose of 5000 IU i.m., 36 h prior to oocyte retrieval. If follicular measurements did not meet the criteria described above then a further scan was performed after 48 h, or a projected follicular growth of ~2 mm/day was employed to predict the time of HCG injection. Transvaginal oocyte retrieval under ultrasound guidance was performed 36 h post HCG. Once identified the oocyte was placed in 1 ml culture medium i.o.(m)., 36 h prior to oocyte retrieval. If follicular measurements did not meet the criteria described above then a further scan was performed after 48 h, or a projected follicular growth of ~2 mm/day was employed to predict the time of HCG injection. Transvaginal oocyte retrieval under ultrasound guidance was performed 36 h post HCG. Once identified the oocyte was placed in 1 ml culture medium (bicarbonate-buffered Earle’s balanced salt solution + 0.03 M sodium pyruvate + 15% human serum albumin + penicillin + gentamycin), in a Nunclon 4-well dish (Nunc, Copenhagen, Denmark) and incubated in a humid atmosphere at 37°C, with 5% CO2. The oocytes were inseminated 40–42 h post HCG with ~100 000 spermatozoa per well. The appearance of pronuclei was checked 16–20 h post insemination. Embryo transfer was performed on day 2 when the embryos were at the 2–4-cell stage. Luteal support was achieved with either HCG (Pregnyl) 2500 IU on the day of transfer and 72 h later, or daily i.m. administration of 100 mg progesterone (Gestone; Paines & Byrne, Surrey, UK). In this unit we administer luteal support with GnRHa cycles based on risk assessment of ovarian hyperstimulation syndrome. Patients who recruit >20 follicles or who have >15 oocytes collected are considered at high risk and are given i.m. progesterone for luteal support, whereas those with fewer follicles and eggs receive i.m. HCG. Pregnancy tests were performed on day 14 (HCG support) or day 16 (progesterone support) post embryo transfer, depending on the method of luteal support, using a commercial urinary kit. Clinical pregnancies were confirmed by transvaginal ultrasound scan at 6–7 weeks gestation. A venous blood sample was taken from each patient on the first day of GnRH administration and every third day of the down-regulation and stimulation phases of the cycle, up until the day of HCG administration. These samples were analysed for serum concentrations of LH, follicle stimulating hormone (FSH), oestradiol and progesterone.

Hormonal assays

Serum FSH and LH were measured in all samples using a Technicon Immuno 1 automated clinical analyser employing a sandwich immunoassay format, with a detection limit of 0.1 mIU/l. Progesterone and oestradiol assays used a similar kit employing a competitive immunoassay format with a detection limit of 0.4 nmol/l and 37 pmol/l respectively. Serum HCG concentrations were determined with a commercial kit (AXSYM; Abbot Laboratories, Abbot Park, IL, USA) based on microparticle enzyme immunoassay (MEIA) technology. Sensitivity of the assay was ≥ 2.0 mIU/ml.

Statistical analysis

All data were expressed as mean ± SEM, and analysed using SPSS version 7.0. One-way analysis of variance (ANOVA), or multiple ANOVA (MANOVA) were used to assess results. Statistical significance was defined as P < 0.05 for all tests.

Results

Statistical analysis showed no significant difference in the mean age of patients in the three groups, A, B or C (30.9, 32.4, 30.8 years respectively), nor in the mean gonadotrophin stimulation dose (42.6, 48.6, 42.1 ampoules respectively).

Figure 1a–d, show serum hormone concentrations of oestradiol, FSH, LH and progesterone, expressed as mean ± SEM against time (days), in each group, in the down-regulation phase of the cycle. The curve for each group was analysed independently and then compared at each time-point with the curves for the other two groups. Cut-off limits for satisfactory pituitary/ovarian suppression were set at 2.5 U/L, 0.5 U/L, 130 pmol/l and 2 mmol/l for FSH, LH, oestradiol and progesterone respectively. There was no significant difference between the curves for FSH, LH or progesterone. However, for oestradiol (Figure 1a), the suppression curve of oestradiol with buserelin was significantly different (P < 0.05) when compared with the curves for nafarelin or leuprolerin. Patients on buserelin took longer for their oestradiol to be suppressed to the cut-off limit of 130 pmol/l, though by day 21 of the down-regulation phase, all three analogues had achieved comparable suppression.

Table I shows the percentage reduction in serum concentrations of oestradiol from initial values to the defined cut-off limit. All three analogues achieved comparable reduction of oestradiol by day 21 of the cycle. However, on day 6 and day 15 there was a significant difference in the percentage reduction of oestradiol when patients using buserelin were compared with patients on nafarelin or leuprolerin (25 versus 44 and 52% respectively; P < 0.05) (54 versus 72 and 65% respectively; P < 0.05). Furthermore, by day 15, 95% of patients using nafarelin or leuprolerin had suppressed oestradiol to <130 pmol/l, compared with only 80% of patients using buserelin (P < 0.05) (Table II).

Figure 2a–d shows the effect of the agonists on serum concentrations of FSH, LH, oestradiol and progesterone, during the stimulation phase of the cycle. There was no significant difference in escape from suppression between the three analogues, as indicated by continually suppressed serum LH concentrations, nor was there any evidence of profound suppression in any of the groups as shown by poorly rising or depressed serum concentrations of oestradiol.

The clinical result of IVF treatment cycles is compared in Table III. Gonadotrophin dose (ampoules) was similar in all groups, as was the duration of stimulation. There was no significant difference between the analogues in follicular recruitment, numbers of oocytes retrieved and fertilized, nor in the number of embryos formed, cleaved or transferred.

Figure 3 shows the implantation, pregnancy and miscarriage rates in patients in the three groups, with no significant difference demonstrated in any of the parameters investigated.

Discussion

A number of studies investigating GnRH analogues have compared their use in various regimes. The ‘ultrashort’ and ‘short’ protocols employ analogues commenced at the start of the menstrual cycle and continued for 3 days with the ultrashort regime and until the day of HCG administration with the short protocol. Thereafter multiple follicular development is achieved with gonadotrophins. These two regimes utilize the
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Figure 1. (a) The effect of nafarelin, buserelin and leuprorelin on serum oestradiol in the down-regulation phase. (b) The effect of nafarelin, buserelin and leuprorelin on serum follicle stimulating hormone (FSH) in the down-regulation phase. (c) The effect of nafarelin, buserelin and leuprorelin on serum luteinizing hormone (LH) in the down-regulation phase. (d) The effect of nafarelin, buserelin and leuprorelin on progesterone in the down-regulation phase.

Table I. Percentage reduction of oestradiol, on days 6, 15 and 21 achieved by nafarelin, leuprorelin and buserelin

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<thead>
<tr>
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<th>Days from start of menstrual period</th>
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<tr>
<td></td>
<td>6</td>
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<tr>
<td>Nafarelin</td>
<td>44</td>
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<tr>
<td>Leuprorelin</td>
<td>52</td>
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<tr>
<td>Buserelin</td>
<td>25</td>
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<td><em>P</em> value</td>
<td>&lt; 0.05</td>
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Table II. Percentage of patients down-regulated on days 12, 15 and 21 after treatment with nafarelin, leuprorelin or buserelin

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<th>Days from start of menstrual period</th>
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<tr>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Nafarelin</td>
<td>90</td>
</tr>
<tr>
<td>Leuprorelin</td>
<td>80</td>
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<tr>
<td>Buserelin</td>
<td>68</td>
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<td><em>P</em> value</td>
<td>&lt; 0.05a</td>
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NS = not significant.
*a* Comparing buserelin with nafarelin and leuprorelin.

initial stimulatory effect of endogenous, stored, and subsequently released pituitary gonadotrophins, as an adjunct to HMG for follicular growth (Tan, 1996). The long protocol of down-regulation, however, allows for complete suppression of oestradiol before ovarian stimulation is begun. This suppression process may take at least 14 days (Neveu et al., 1987; Kerin, 1989). Although some authors have found no difference in clinical outcome between the long and short protocols, others including Kerin (1989), Smitz et al. (1992), Tarlatzis et al. (1994a) and Tan et al. (1996) have shown higher implantation and pregnancy rates with the long protocol. This has been attributed to more effective LH suppression, higher oocyte retrieval per cycle, more embryos developed and a higher chance of pregnancy from the use of cryopreserved embryos.
GnRH analogues for IVF

Figure 2. (a) The effect of nafarelin, buserelin and leuprorelin on serum oestradiol in the stimulation phase. (b) The effect of nafarelin, buserelin and leuprorelin on serum follicle stimulating hormone (FSH) in the stimulation phase. (c) The effect of nafarelin, buserelin and leuprorelin on serum luteinizing hormone (LH) in the stimulation phase. (d) The effect of nafarelin, buserelin and leuprorelin on serum progesterone in the stimulation phase.

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<th>Table III. Clinical results after treatment with nafarelin, buserelin and leuprorelin (all mean values ± SD)</th>
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<td></td>
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<tr>
<td>No. of cycles</td>
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<td>Incidence (%) of cysts &lt;20 mm</td>
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<tr>
<td>No. of HMG ampoules</td>
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<tr>
<td>Day of HCG</td>
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<td>No. of follicles</td>
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<td>No. of oocytes</td>
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<td>No. of viable oocytes</td>
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<tr>
<td>No. of oocytes fertilized</td>
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<tr>
<td>No. of embryos</td>
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<tr>
<td>No. of embryos cleaved</td>
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<td>No. of embryos transferred</td>
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| Figure 3. Implantation, pregnancy and miscarriage rates after treatment with the three analogues, nafarelin, buserelin and leuprorelin. |

There were no significant differences between groups.

HMG = human menopausal gonadotrophin; HCG = human chorionic gonadotrophin.

in the same cycle. The long protocol of down-regulation initiated in the mid-luteal phase produces more prompt and profound suppression with a lower resultant incidence of cyst formation (Martin et al., 1992), by reducing the flare response (Lockwood et al., 1995). An incidence of ~5% cyst formation has been found in IVF cycles and possibly higher if down-regulation is started in the early follicular phase (Jenkins, 1996). However, studies comparing ovarian cysts in IVF cycles pretreated with GnRHα are plagued by problems of definition and true influence, with only steroidically active cysts being found detrimental to IVF outcome (Jenkins et al., 1992).
Nevertheless, Serafini et al. (1988a), Meldrum et al. (1988), Ron El et al. (1990b) and Daya (1993), comparing the long protocol commenced in the mid-luteal phase with early follicular phase starts, have shown higher follicular recruitment, oocyte retrieval and pregnancy rates with follicular protocols of GnRH agonists administration. Since the establishment of this unit in 1991, we have utilized early follicular phase administration of GnRH agonists with good results (Assisted Conception Unit, 1997).

Our results agree with those of Penzias et al. (1992), Balasch et al. (1992), Tarlatzis et al. (1994a) and Porcu et al. (1995), i.e. long-acting depot preparations are as effective in pituitary suppression as the short-acting analogues. We found no significant difference in suppression of FSH, LH, oestradiol or progesterone with any of the three analogues after 21 days of administration. We also found that the number of patients suppressed as well as the percentage suppression of serum oestradiol to defined cut-off values, by day 15, was superior with nafarelin or leuprorelin when compared to patients on buserelin. The incidence of cyst formation in all three groups was similar (nafarelin = 10%; buserelin = 10%; leuprorelin = 15%). Tarlatzis et al. (1994b) have shown that cyst formation with GnRH agonists is associated with elevated serum oestradiol concentrations. The delayed suppression with persistence of cysts observed with the patients on buserelin could indicate failure of buserelin to deal effectively with this adjunctive oestrogen production. We found no evidence of escape from suppression with any of the three analogues, comparing the number of viable oocytes retrieved or fertilized. In addition, leuprorelin did not cause profound suppression by comparable gonadotrophin dose and duration of stimulation.

One of the recognized complications of analogue use in a long protocol is the risk of luteal phase dysfunction from premature luteolysis. Inhibition of luteal steroidogenesis by GnRH agonists has been described by Smitz et al. (1987). Short-acting analogues such as buserelin or nafarelin have been traditionally favoured because of their brevity of action. At cessation of administration they rapidly disappear from the circulation and a normal pituitary response can be shown within a few days (Porcu et al., 1995). Any effect on the luteal phase is thus minimal. Depot preparations have a longer duration of action, ~7 weeks (Broekmans et al., 1992; Porcu et al., 1995), with potentially adverse consequences on corpus luteal function. A report by Bourgain et al. (1994) investigated the influence of GnRH agonists on the endometrium and endometrial development. Endometrial histological maturation, ultrastructure, oestrogen and progesterone receptor status were analysed in the mid-luteal phase in GnRHa/HMG cycles with and without luteal supplementation. They found that supplementation with HCG or progesterone produced fewer signs of luteal phase deficiency compared to non-supplemented cycles. All the patients in this study received luteal phase support with either HCG or progesterone, and we found no difference in the miscarriage rates of patients on leuprorelin as compared with either nafarelin or buserelin. Filiconi et al. (1996) have noted that depot GnRHa administration that provides measurable drug concentrations in early pregnancy is not associated with an increased abortion rate. Moreover we did not find any difference in implantation rates or pregnancy rates between the three agonists. Although the presence of GnRH and its receptor has been established in endometrium, until recently it has been unknown whether these receptors are present on preimplantation embryos. Inadvertent exposure of concepti to GnRHa does not appear to have a deleterious effect (Tolis et al., 1981; Skarin et al., 1982; Testart et al., 1993). Gartner et al. (1997) have even suggested that analogues may be beneficial to implantation. A recent study by Raga et al. (1998) has demonstrated the presence of GnRH receptors on preimplantation embryos at the mRNA and protein levels. Their randomized study between administration or omission of GnRHa until 6 weeks of gestation, with luteal support, showed that administration of GnRHa throughout the luteal phase and early pregnancy had a positive influence on pregnancy and implantation outcome. Direct effects of long-acting depot preparations on the embryo can now be viewed in a fuller context and seem unlikely to be detrimental. Furthermore we noted no difference in the mean numbers of embryos formed or cleaved in those patients on leuprorelin compared to the shorter acting analogues.

Our study shows that leuprorelin, nafarelin and buserelin are equally effective when pituitary suppression, ovarian stimulation and IVF outcome are compared. Although satisfactory suppression with nafarelin and leuprorelin occurred after 2 weeks, buserelin required an additional week to achieve equivalent results. However, after 3 weeks there was no significant difference between the three analogues.

GnRH antagonists, particularly the cyclic compounds which have a potency comparable with the linear agonists, may become more widespread in general clinical use. Their advantages include no initial flare response due to an immediate competitive pituitary receptor occupancy (Rivier et al., 1996). Further work in rat models may ultimately lead to the availability of GnRH antagonists with oral activity and minimal histamine-releasing side-effects (Rivier et al., 1996), making them eminently more attractive.

At present, however, the decision on which GnRH agonist to use will ultimately depend on patient and clinician preference, side-effects, availability and cost.

References


Received on March 27, 1998; accepted on October 28, 1998


Received on March 27, 1998; accepted on October 28, 1998