A randomized prospective study of microdose leuprolide versus ganirelix in in vitro fertilization cycles for poor responders

Forty-eight patients who met the criteria of poor response during prior gonadotropin stimulation were enrolled in a randomized prospective study comparing a gonadotropin-releasing hormone (GnRH) antagonist protocol, using ganirelix acetate, with a microdose GnRH agonist protocol for in vitro fertilization-embryo transfer (IVF-ET). This pilot study contributes to the literature of poor response IVF treatment protocols because the use of ganirelix appears to be as effective as the microdose protocol and may be a superior choice in terms of cost and convenience for the patient. (Fertil Steril® 2005;83:1568–71. ©2005 by American Society for Reproductive Medicine.)

Infertility patients who demonstrate a diminished ovarian response to controlled ovarian hyperstimulation (COH) are challenging to treat. One protocol for poor responders is the microdose flare protocol (1, 2). This protocol takes advantage of the initial rise in endogenous gonadotropins that follows gonadotropin-releasing hormone agonist (GnRH-a) administration in the early follicular phase and maintains efficacy in premature luteinizing hormone (LH) surge prevention. Pretreatment with oral contraceptives prevents corpus luteum formation and subsequent stimulation from exogenous GnRH-a (2). The microdose flare protocol has been proven to improve cycle parameters, decrease cancellation rates, and increase both clinical and ongoing pregnancy rates in poor responders (2, 3).

The availability of effective GnRH antagonists has recently offered an alternative protocol for poor responders. Because the antagonist can be administered in the late follicular phase for premature LH surge prevention, suppression in the early follicular phase is avoided (4). The purpose of this pilot study was to compare the microdose flare protocol with the GnRH antagonist protocol, using ganirelix acetate in poor responder patients in a randomized prospective fashion.

Patients who had previously shown a poor response to gonadotropin stimulation at the Center for Advanced Reproductive Services at the University of Connecticut Health Center were enrolled between November 2000 and May 2003 and underwent treatment after randomization. A computer-generated randomization was used for assignment of the two treatment protocols with a 1:1 ratio, distributed in a block design with six patients per block. Sealed envelopes were used for protocol allocation with each patient to prevent selection bias. Informed patient consent was obtained before entry into the study, which was approved by the Institutional Review Board of the University of Connecticut Health Center.

A total of 48 patients with poor ovarian responses during stimulation in previous assisted reproduction cycles at our center participated in the study. Patients undergoing IVF treatment were between the ages of 25 and 43 years. Poor ovarian response was defined as a serum peak estradiol (E2) level ≤850 pg/mL (conversion factor to international System of Units [SI] unit, 3.67) and/or ≤4 preovulatory follicles ≥15 mm in average diameter present on the day of human chorionic gonadotropin (hCG) administration during a previous cycle of COH. Prior stimulation regimens consisted of 300 IU of gonadotropins with or without leuprolide acetate pituitary down-regulation. Patients were required to have a cycle day 3 serum follicle-stimulating hormone (FSH) concentration <13 mIU/mL (conversion factor to SI unit, 1.00) and a serum E2 level <75 pg/mL. Baseline transvaginal sonography was performed on cycle day 2, and no follicular development, defined as a follicle >10 mm, could be present at the start of gonadotropin stimulation.

During the course of the study, patients were randomized to one of two groups: the ganirelix acetate group (group A; n = 24) or the microdose leuprolide flare group (group B; n = 24). Demographic parameters including mean age, height, weight, body mass index (BMI), duration of infertility, basal FSH, and basal E2 levels were comparable in both groups. All patients were evaluated on cycle day 2 with transvaginal ultrasonography and serum E2 levels for an initial assessment before gonadotropin stimulation. Similar evaluations occurred on the 6th day of gonadotropin stimulation.

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stimulation, on the day of hCG administration, and as dictated by ovarian response during treatment. The E2 and progesterone (P) levels were obtained the morning following hCG administration.

After baseline inclusion criteria were met on cycle day 2, patients randomized to group A received 300 IU of recombinant FSH (Follistim®; Organon Pharmaceuticals, Inc., West Orange, NJ) subcutaneously (SC) every morning and 150 IU of human menopausal gonadotropin (hMG) (Repronex®; Ferring Pharmaceuticals, Inc., Suffern, NY) SC every evening for 5 days. The medication regimen was maintained daily with individual adjustment according to ovarian follicular response and serum E2 concentrations. Ganirelax was administered at a dose of 250 μg SC when an E2 concentration ≥250 pg/mL and follicle(s) ≥12 mm in average diameter were observed. A multidose regimen of ganirelax was used with daily morning injections until, and including, the day of hCG administration. Serum LH levels were monitored starting from day 6 of stimulation to exclude a premature surge (LH levels were monitored starting from day 6 of stimulation to exclude a premature surge (LH ≥15 mIU/mL).

Those patients randomized to group B received 21 days of oral contraceptive (OC) pills (Desogen; Organon Pharmaceuticals, Inc.) beginning on day 3 of their menses preceding the treatment cycle. Baseline assessment was performed identical to group A on the 3rd morning following the patient’s last OC. If all inclusion criteria were met, leuprolide acetate (Lupron®; TAP Pharmaceuticals, North Chicago, IL) was begun with a regimen of 40 μg SC administered every 12 hours (bid). Serum E2, FSH, and LH levels were obtained 2 days following the initiation of leuprolide acetate, and gonadotropins were begun that day. The FSH and hMG dosing, monitoring, and individualized dosing adjustments were the same as group A.

In both groups, a total of 10,000 IU of hCG were administered intramuscularly (IM) for ovulation induction when at least three follicles of 17 mm in diameter and E2 levels ≥850 pg/mL were obtained. Failure to achieve these criteria after 10 to 12 days of stimulation resulted in cycle cancellation for inadequate response. Oocyte retrieval was performed 35 hours after hCG administration by transvaginal ultrasound-guided puncture of follicles. The IVF and intracytoplasmic sperm injection (ICSI) procedures were performed as previously described (5, 6). Embryos were transferred 3 days following oocyte retrieval. The number of embryos transferred varied according to the patient’s age and embryo quality. All patients received progesterone in oil (50 mg/d) IM, which was initiated the day after retrieval until a negative serum pregnancy test (βhCG) was achieved. If the test was positive (βhCG >5 IU/L), a repeat value was obtained 48 hours later; an adequate rise of serum βhCG defined a positive pregnancy, and the progesterone support was continued. A clinical pregnancy was defined as a normal gestational sac measured with transvaginal ultrasonography after 5 weeks, and an ongoing pregnancy was defined as a clearly visible fetal pole with a normal fetal heart rate observed after 8 weeks.

The primary outcome measures were: total dose of gonadotropin administered, days of stimulation, cancellation rates, number and size of follicles, endometrial thickness, E2 levels at the time of hCG administration as well as peak E2 concentrations, total number of oocytes retrieved, number of mature oocytes (as assessed in patients utilizing ICSI), quality of developed embryos, implantation, and ongoing pregnancy rates. Demographics for both groups were compared as well as the number of embryos transferred in each group using the Student’s t-test. Statistical analysis was performed for clinical outcome variables using the Student’s t-test, χ² test, or Fisher’s exact tests where appropriate, as well as the rank sum test when appropriate for nonparametric data. A P value <.05 was considered statistically significant. All data are expressed as means ± SE, unless stated otherwise.

Demographic parameters including mean age, height, BMI, duration of infertility, basal FSH, and E2 levels were similar between the two groups before the initiation of treatment. No relevant differences were found between the treatment groups for the etiology of infertility, which, overall, included 35.4% tubal factor pelvic adhesions, 20.8% male factor, 16.7% endometriosis, and 27.1% unexplained infertility. The overall percentage of women with primary infertility was 39.6%, which was similar in both treatment groups. The ICSI technique was performed with IVF in approximately 50% of the cases for the indications of male factor infertility or unexplained infertility, and was also distributed evenly between the two groups.

The cancellation rates were similar for group A and group B (41.7% vs. 50%; P=.562). Both protocols were effective in preventing a premature LH surge (LH <15 mIU/mL) in all patients, and cycle cancellations were exclusively due to an inadequate response. On the day of cancellation, the mean peak E2 levels did not differ (541 ± 51 vs. 613 ± 148 pg/mL; P=.667). Canceled patients did have a significantly higher mean basal FSH value compared with patients who completed their cycle treatment (9.3 ± 0.6 vs. 7.2 ± 0.4; P=.006). All other demographic parameters were similar between the cancelled patients and the patients completing treatment. No cases of inadequate endometrial development (<8 mm) were noted in completed or cancelled cycles.

Demographic variables were also similar between the two groups for completed cycle patients. Both stimulation protocols were effective in preventing a premature LH surge. No statistically significant differences were noted between any of the primary outcome parameters for patients in group A or group B who completed their cycle (Table 1). Embryo quality was assessed by zygote scoring (z-score) on day 1 following IVF (7), as well as on day 3 using the criteria of Veeck (8). There was no statistical
difference between the embryo quality in both treatment groups (data not shown).

The purpose of this pilot study was to compare, in a randomized prospective fashion, the microdose leuprolide protocol with a ganirelix protocol in poor responders. The results of our clinical trial in poor responders showed no significant difference for the number of mature follicles, mean number of oocytes, mature oocytes, fertilization rates, implantation rates, or clinical pregnancy rates. This is in accordance with previous studies of GnRH antagonists (9, 10).

Few studies compare the efficacies of the microdose flare protocol with a GnRH antagonist protocol for this population; only one is a randomized prospective trial (11). Akman et al. (11) studied 48 poor responder patients randomized to either a microdose flare protocol or a GnRH antagonist protocol using cetrorelix (0.25 mg/d) in the late follicular phase. As observed in our study, ampules of FSH and hMG used, median number of follicles, and mature oocytes were similar. Fertilization rates, number of embryos transferred, implantation rates, and ongoing pregnancy per transfer did not differ, given the use of a different agonist.

In contrast to our results, Akman et al. (11) found significantly higher E₂ levels on the day of hCG and a greater mean number of total oocytes retrieved in the microdose protocol compared with the GnRH antagonist protocol. Although we also saw a trend of higher E₂ levels (not significant) in the microdose group, our results showed a remarkably similar number of oocytes obtained in both groups and no difference in other IVF outcomes, including embryo quality. The cancellation rates of 20%–25% in Akman’s study were somewhat lower than those observed in our investigation. The difference in our results vs. those of Akman et al. are minor and may be accounted for by the differences in patient population and inclusion criteria, gonadotropin dose given, or subtle differences in the effects of the two different antagonists used (i.e., cetrorelix vs. ganirelix). In addition to the IVF outcome measures assessed by Akman et al., our study also analyzed mean endometrial thickness, as well as embryo quality, with no significant differences noted between the two treatment groups.

The conclusions that can be drawn from these two randomized prospective trials, comparing the microdose flare protocol with a GnRH antagonist protocol in poor responders, are limited by a small sample size. This population of patients typically represents between 5% and 18% of infertile patients presenting for treatment (2, 12). The relatively small population of patients that fit the criteria of a poor responder attests to the difficulty of patient recruit-

### Table 1

<table>
<thead>
<tr>
<th>Outcome for completed patients.</th>
<th>Ganiirelix acetate (n = 13)</th>
<th>Leuprolide acetate (microdose) (n = 11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of stimulation</td>
<td>10.1 ± 0.3</td>
<td>10.1 ± 0.6</td>
<td>.986</td>
</tr>
<tr>
<td>rFSH (IU)</td>
<td>2,760 ± 150</td>
<td>2,685 ± 202</td>
<td>.775</td>
</tr>
<tr>
<td>hMG (IU)</td>
<td>1,230 ± 112</td>
<td>1,350 ± 97</td>
<td>.436</td>
</tr>
<tr>
<td>E₂ at trigger (pg/mL)</td>
<td>1,348 ± 212</td>
<td>1,819 ± 276</td>
<td>.182</td>
</tr>
<tr>
<td>E₂ post-hCG (pg/mL)</td>
<td>1,455 ± 208</td>
<td>2,080 ± 327</td>
<td>.110</td>
</tr>
<tr>
<td>P₄ post-hCG (pg/mL)</td>
<td>3.9 ± 0.7</td>
<td>4.3 ± 0.5</td>
<td>.681</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>10.6 ± 0.5</td>
<td>9.6 ± 0.7</td>
<td>.241</td>
</tr>
<tr>
<td>Follicles &gt;16 mm</td>
<td>4.3 ± 0.3</td>
<td>3.8 ± 0.6</td>
<td>.443</td>
</tr>
<tr>
<td>Follicles 14–16 mm</td>
<td>2.9 ± 0.6</td>
<td>2.6 ± 1.1</td>
<td>.817</td>
</tr>
<tr>
<td>Follicles &lt;14 mm</td>
<td>3.2 ± 0.8</td>
<td>2.3 ± 0.5</td>
<td>.350</td>
</tr>
<tr>
<td>No. of oocytes</td>
<td>8.9 ± 0.9</td>
<td>9.0 ± 1.2</td>
<td>.962</td>
</tr>
<tr>
<td>No. of mature oocytes</td>
<td>7.7 ± 0.9</td>
<td>6.5 ± 1.1</td>
<td>.408</td>
</tr>
<tr>
<td>Fertilization rate⁴</td>
<td>69.1%</td>
<td>63.5%</td>
<td>.530</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>.879</td>
</tr>
<tr>
<td>Clinical pregnancy rate⁵</td>
<td>38.5% (5/13)</td>
<td>36.4% (4/11)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Note: Data expressed as mean ± SEM. Groups compared using Student’s t-test, unless noted; rFSH = recombinant follicle-stimulating hormone; hMG = human menopausal gonadotropin; E₂ = estradiol; hCG = human chorionic gonadotropin; P₄ = progesterone.

⁴ χ² test.
⁵ Fisher’s exact test.

ment and limited statistical power of these results. To exclude a 10% difference in pregnancy rates between the two groups in the current investigation, the study would require a sample size of 632 patients (assuming \( \alpha = 0.05 \) and \( \beta = 0.20 \)). Thus, we cannot rule out the possibility of type II statistical errors in our results.

This is the first randomized prospective study to compare the GnRH antagonist stimulation protocol using ganirelix acetate to the microdose flare protocol in poor responder patients undergoing COH for IVF. Optimal stimulation of the poor responder remains a challenge. This group of patients has traditionally shown poor IVF outcomes regardless of the treatment protocol. Our findings demonstrate that the use of ganirelix for GnRH antagonist protocols appears to be as effective as the conventional microdose protocol in the treatment of poor responders. Not only were IVF cycle outcomes similar, but embryo quality, implantation rates, and ongoing pregnancy rates were comparable. Selection of either protocol appears reasonable for these patients, yet the GnRH antagonist protocol may be preferable because it requires significantly fewer injections of the GnRH antagonist in comparison with the agonist and a shorter treatment course because pretreatment with the oral contraceptive is not used.

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