Comparison of daily and alternate day recombinant follicle-stimulating hormone stimulation protocols for intrauterine insemination

Nadia Kabli, M.D., Camille Sylvestre, M.D., Togas Tulandi, M.D., MHCM, and William Buckett, M.D.

Department of Obstetrics and Gynecology, McGill University, Royal Victoria Hospital, Montréal, Québec, Canada

Objective: To determine the efficacy of daily versus alternate-day stimulation for patients undergoing intrauterine insemination (IUI).

Design: Retrospective comparative study.

Setting: University tertiary care center for infertility.

Patient(s): Women with anovulatory or unexplained infertility for over 12 months who had not responded to or not conceived with clomiphene citrate treatment.

Intervention(s): Ovarian stimulation with recombinant follicle-stimulating hormone (FSH; follitropin-beta) given either daily or on alternate days before IUI.

Main Outcome Measure(s): Clinical pregnancy at ultrasound 4 weeks after IUI. Secondary outcome measures were duration of stimulation, total gonadotropin dose, ovarian response, and multiple pregnancy rate.

Result(s): Of the 212 women who underwent a first cycle of gonadotropin stimulation, 28 had daily injections and 117 had alternate-day injections of follitropin-beta only for IUI. Female age, antral follicle count, and day-3 serum FSH were comparable. The median duration of stimulation (8 days vs. 8 days) and the median number of follicles over 14 mm (3.5 vs. 3.0) were similar in both groups. However, the total recombinant FSH dose (825 vs. 625 IU) and endometrial thickness (10.1 vs. 9.3 mm) were greater in the daily injection group. The clinical pregnancy rate per cycle was 42% (12 out of 28) in the daily injection group and 19% (22 out of 117) in the alternate-day group.

Conclusion(s): Daily recombinant FSH stimulation for IUI seems to be associated with a higher clinical pregnancy rate than alternate-day FSH stimulation. Prospective randomized trials would be needed to determine whether this is indeed the case. (Fertil Steril® 2009;91:1141–4. ©2009 by American Society for Reproductive Medicine.)

Key Words: Follitropin-beta, IUI, ovarian stimulation, recombinant FSH

The empiric practice of superovulation and intrauterine insemination (IUI) has been successfully used as a treatment for unexplained infertility and refractive anovulatory infertility for over 20 years. Although studies have been conflicting, meta-analyses have proven the approach to be effective at increasing pregnancy and live-birth rates (1–3). Superovulation with daily injections of gonadotropins, in the form of human menopausal gonadotropin (hMG), were reported in the 1970s (4, 5), and most reported ovarian stimulation protocols for IUI today—whether with the use of urinary follicle-stimulating hormone (FSH), recombinant FSH, or hMG—also use daily injections (6–9). However, the use of alternate day hMG was originally reported in 1988 (10), and several studies continue to use this form of protocol for superovulation in couples undergoing IUI (11–13). The perceived benefits of alternate-day injection include halving the number of injections for the woman and lessening the expense via half-am- pules of gonadotropin.

However, to our knowledge, no studies have ever compared the efficacy of daily with alternate-day injection—whether with hMG or more recently with either urinary FSH or recombinant FSH. Therefore, the objective of this study was to compare the efficacy of daily injections versus alternate day injections of a single recombinant gonadotropin (follitropin-beta) in couples undergoing a first cycle of IUI.

MATERIALS AND METHODS

Retrospective analysis of all superovulation and IUI cycles during the period from January 2005 to December 2006 was carried out. Inclusion criteria were all couples with infertility after ≥1 year of unprotected intercourse with either a diagnosis of unexplained infertility or with anovulatory infertility where they had not conceived after three cycles of ovulation induction with clomiphene citrate. The women’s age was <40 years.

The diagnosis of unexplained infertility was made by normal early follicular phase ultrasound (no cysts, no endo-metrioma, no fibroids) and normal serum FSH and LH (<10 IU/L). Midluteal serum progesterone was >20 nmol/
L. Hysterosalpingogram (HSG) revealed patent tubes and a normal uterine cavity. Sperm concentration was >20 million/mL with >40% progressively motile and <40% abnormal morphology. Anovulatory infertility allowed for polycystic ovaries on early follicular phase ultrasound and an LH level >10 IU/L, but invariably had a midluteal phase serum progesterone <20 nmol/L. All cases of anovulatory infertility had a normal HSG and seminal analysis as detailed earlier.

**Superovulation**

Initial stimulation dose of subcutaneous gonadotropin follicle-stimulating hormone (Puregon; Organon, Oakville, Ontario, Canada) was calculated according to female age, level of serum FSH, the diagnosis of polycystic ovary syndrome, and weight. Typically, the starting dose was either 150 IU or 225 IU on day 3, day 5, day 7, and day 9 of the cycle in the alternate-day injection group and 75 IU or 100 IU from day 3 in the daily injection group. The injections were started after the day-2 or day-3 ultrasound had excluded the presence of an ovarian cyst.

All the patients were reevaluated on day 9 for quality of the response, and the dose was increased by the equivalent of 75 IU/day if there was no follicle over 12 mm mean diameter. Stimulation continued until one to three follicles reached a size of ≥16 mm with a leading dominant follicle size of ≥18 mm. Then 10,000 IU human chorionic gonadotropin (hCG) was given subcutaneously, and the IUI was performed at 24 and 48 hours later.

A serum pregnancy test was performed 2 weeks later, and an intrauterine pregnancy was confirmed by detection of a gestational sac using transvaginal echography 4 weeks after the insemination.

**Semen Preparation and Insemination Technique**

Semen samples were collected in sterile containers and were processed by washing and swim-up technique (13). A 0.5-mL washed sample was injected into the uterine cavity using a Rocket IUI catheter (Rocket Medical, Watford, United Kingdom). Patients were then advised to remain in supine position for 15 minutes after insemination.

**Cycle Cancellation**

The ovarian stimulation cycle was canceled after evidence of ovarian overresponse. This was defined as a growth of ≥4 follicles of ≥16 mm mean diameter. Furthermore, if there was no follicular development after day 21 of the cycle, the cycle was similarly canceled.

**Outcomes**

The main outcome measure was clinical pregnancy at ultrasound 4 weeks after insemination. Secondary outcome measures were the duration of stimulation, total gonadotropin dose, ovarian response, endometrial thickness, cycle cancellation rate, and multiple pregnancy rates.

**Statistical Analysis**

The data obtained were analyzed using StatsDirect software (StatsDirect Ltd, Altrincham, Cheshire, United Kingdom). Results were analyzed following the intention-to-treat principle. Categorical data were compared using chi-square test and Fisher’s exact test. Continuous data were compared using the unpaired t-test for normally distributed data, and Mann-Whitney U test for non-normally distributed data. The Shapiro-Wilks test was used to determine normality. P<.05 was considered statistically significant.

Sample size calculation is 121 subjects, assuming a pregnancy rate of 15% in the alternate day stimulation group and 30% in the continuous day stimulation, with 80% power for a 5% chance of incorrectly rejecting the null hypothesis (alpha).

Ethics committee approval was not sought for this specific study as it is a review of data already collected. Local institutional review board rules determine that approval from the institution’s Director of Professional Services (DPS) is needed, which was granted.

**RESULTS**

During the period from January 2005 to December 2006, a total of 326 IUI cycles were performed, and 212 cycles were a first IUI cycle. The women received a variety of gonadotropins (follitropin-beta 145; follitropin-alpha 63; hMG 4). Of those who used only follitropin-beta, 28 had daily injections, and 117 had alternate-day injections. There was no difference in age, early follicular phase FSH level, antral follicle count, incidence of PCOS, or incidence of primary infertility between the two groups, although the starting dose of FSH, as expected, was lower in the daily group (Table 1).

Clinical pregnancy was statistically significantly higher in the daily injection group (42%) compared with the alternate-day injection group (19%). The relative risk (RR) for pregnancy was 2.28 (95% CI, 1.26–3.91) in favor of the daily group (Table 2).

Similarly, the endometrial thickness on the day of hCG and the total dose of follitropin-beta was greater in the daily injection group. Although the median number of follicles >14 mm was higher in the daily injection group (3.5 vs. 3.0), this difference did not reach statistical significance. However, the incidence of twin pregnancy was higher in the daily injection group. There were no triplet or higher order pregnancies. Cycle cancellation was 4% in the alternate-day injection group and 0 in the daily injection group (see Table 2).

When the data for both follitropin-beta and follitropin-alpha are combined, the relative risk of clinical pregnancy was 2.41 (95% CI, 1.13–6.17) in favor of the daily injection group (11 out of 41 cycles) and against the alternate-day injection group (22 out of 167). The post hoc approximate power was 60% (for an alpha of 5%).
DISCUSSION

Alternate-day gonadotropin injection protocols have been used widely over the past two decades; however, to our knowledge, no study has directly compared daily injections with alternate-day injections. Although this is a retrospective study and thus has all the appropriate caveats, by limiting the analysis to unexplained infertility and clomiphene-resistant anovulatory infertility and only assessing first IUI cycles with a single gonadotropin preparation (follitropin-beta), an attempt has been made to compensate for some of the confounding variables. Other confounding variables undoubtedly exist, but it would appear that daily injections are superior to alternate-day injection.

In the past, reasons for preferring alternate-day gonadotropin were that of reducing the burden of intramuscular injection—for example, from eight to four injections per cycle. However, since the advent of high-purity urinary gonadotropins and, more recently, recombinant gonadotropins, subcutaneous administration has become the norm (14, 15). Further studies also showed, even with hMG, that subcutaneous injection was as effective as intramuscular injection (16, 17). Therefore, the additional burden of daily injections compared with alternate-day injection has been less marked than in the 1980s.

Another possible advantage of alternate-day injections was that if a half ampule (typically 37.5 IU) was required, if

### TABLE 1

Demographic data.

<table>
<thead>
<tr>
<th></th>
<th>Daily injections (n = 28)</th>
<th>Alternate-day injections (n = 117)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>35.5 (33.8–37.3)</td>
<td>35.8 (34.9–36.7)</td>
<td>.78</td>
</tr>
<tr>
<td>Mean serum FSH (IU/L)</td>
<td>6.47 (5.49–7.53)</td>
<td>6.51 (6.04–6.92)</td>
<td>.95</td>
</tr>
<tr>
<td>Median antral follicle count (interquartile range)</td>
<td>14.5 (11–28.5)</td>
<td>17 (12–29)</td>
<td>.59</td>
</tr>
<tr>
<td>Proportion with PCOS (%) (95% CI)</td>
<td>28 (13–48)</td>
<td>33 (25–42)</td>
<td>.42</td>
</tr>
<tr>
<td>Proportion with primary infertility (%) (95% CI)</td>
<td>66 (48–84)</td>
<td>68 (59–77)</td>
<td>.68</td>
</tr>
<tr>
<td>Median starting dose (IU) (interquartile range)</td>
<td>100 (100–200)</td>
<td>150 (100–200)</td>
<td>.04</td>
</tr>
</tbody>
</table>


### TABLE 2

Outcome data.

<table>
<thead>
<tr>
<th></th>
<th>Daily injections (n = 28)</th>
<th>Alternate-day injections (n = 117)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancy rate (%) (95% CI)</td>
<td>12/28 (42%) (25–63)</td>
<td>22/117 (19%) (12–27)</td>
<td>.01</td>
</tr>
<tr>
<td>Median days of stimulation (interquartile range)</td>
<td>8 (6–10)</td>
<td>8 (6–10)</td>
<td>.81</td>
</tr>
<tr>
<td>Median total FSH dose (IU) (interquartile range)</td>
<td>825 (600–1200)</td>
<td>625 (500–850)</td>
<td>.02</td>
</tr>
<tr>
<td>Median follicles &gt;14 mm (interquartile range)</td>
<td>3.5 (1–5.5)</td>
<td>3 (1–4)</td>
<td>.22</td>
</tr>
<tr>
<td>Mean endometrial thickness (mm) (95% CI)</td>
<td>10.1 (9.3–11.1)</td>
<td>9.3 (8.9–9.7)</td>
<td>.05</td>
</tr>
<tr>
<td>Cancellation rate (%) (95% CI)</td>
<td>0 (0–9)</td>
<td>4 (0–9)</td>
<td>.27</td>
</tr>
<tr>
<td>Twin pregnancy (%) (95% CI)</td>
<td>2/12 (17%) (2–48)</td>
<td>1/22 (5%) (0–23)</td>
<td>.24</td>
</tr>
</tbody>
</table>

would be easier and cheaper (by avoiding throwing away the remaining unused half ampule) to give 75 IU on alternate days. However, opened ampules can be kept overnight in the refrigerator without any detriment to efficacy, although concerns regarding absolute sterility remain. The advent of multidose-pen preparations has made this concern obsolete (18, 19).

In our study, the daily injection group did receive a higher total dose of gonadotropin, and it may be that the increased clinical pregnancy rate associated with daily gonadotropin injection is mediated by this higher dose, particularly as the follicular response, endometrial response, and multiple pregnancy rate were all higher in the daily injection group. There are also pharmacodynamic advantages of daily follitropin-beta injection compared with alternate-day injections. Peak serum concentration is reached after 11 to 13 hours, and the elimination half-life is about 34 hours (18). The terminal half-life with injected gonadotropins generally is 43 to 48 hours after injection (20).

A less steady state is achieved with alternate-day injection when compared with daily injection, although the clinical significance of this has not been studied. This lesser steady state may account for the poorer outcomes in the alternate-day injection stimulation group. However, as already noted, the median overall dose was lower in this group, and the median number of follicles greater than 14 mm was also lower. Therefore, the lower pregnancy rate could also be attributed to these factors.

As noted at the beginning of the discussion, this is a retrospective study. Although the cases were first cycles, with a single recombinant FSH, and all had clomiphene-resistant anovulatory infertility, many other possible biases are present. Not least that, historically, alternate-day injection was the standard of care at our institution, which accounts for the imbalance in the distribution. Furthermore, there may have been unintentionally closer monitoring of the daily injection group. Finally, physician preference could also cause further bias. To determine whether daily gonadotropin injection is genuinely superior to alternate-day gonadotropin injection for superovulation in couples undergoing IUI, an appropriately powered, prospectively randomized, controlled trial would be needed. Given that daily gonadotropin injection is widely accepted worldwide (5) and the advantages of alternate-day gonadotropin are minimal, it is unlikely that such an randomized controlled trial would receive a high priority. Therefore, in the absence of any higher level evidence, superovulation for IUI with injected gonadotropins should be by daily rather than alternate-day injection.

REFERENCES