Safety and efficacy of mixing cetrorelix with follitropin alfa: a randomized study

Yu-Hung Lin, M.D.,[a,b,c] *Yeong-Ray Wen, M.D.,*[d,e] *Yuanmay Chang, R.N., Ph.D.,*[e,f] *Kok-Min Seow, M.D., Ph.D.,*[a,b,g] *Bih-Chwen Hsieh, M.D.,*[a,b] *Jiann-Loung Hwang, M.D.,*[a,e] *and Chii-Ruey Tzeng, M.D.*[e]

[a] Department of Obstetrics and Gynecology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan; [b] School of Medicine, Fu Jen Catholic University, Taipei, Taiwan; [c] Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan; [d] Department of Anesthesiology, Shin Kong Wu Ho-Su Memorial Hospital; [e] School of Medicine, Taipei Medical University, Taipei, Taiwan; [f] Department of Nursing, Shin Kong Wu Ho-Su Memorial Hospital; and [g] Department of Obstetrics and Gynecology, Li Shin Hospital, Taoyuan, Taiwan, Republic of China

Objective: To assess the safety and efficacy of mixing cetrorelix with follitropin alfa (rFSH) in assisted reproductive technology.
Design: Prospective, randomized study.
Setting: An IVF center in a teaching hospital.
Patient(s): One hundred forty patients undergoing intracytoplasmic sperm injection were randomized into mixed (M) or separate (S) injection groups.
Intervention(s): In the M group, rFSH and cetrorelix were mixed immediately before administration, whereas in the S group, rFSH and cetrorelix were administered separately.
Main Outcome Measure(s): The primary efficacy end point was the incidence of premature LH surge. The secondary efficacy endpoints included estradiol levels on the day of hCG injection, numbers of oocytes obtained, implantation, and ongoing pregnancy rates. The safety endpoints included ovarian hyperstimulation syndrome, and adverse events related to injections including local tolerability.
Result(s): Excluding eight patients who dropped out of the study, there were 66 patients in each group for analysis. Patients in the M group received significantly fewer injections than patients in the S group (9.1 vs. 13.9). Other outcome parameters, including incidences of premature LH surge, numbers of oocytes retrieved, fertilization, implantation, and ongoing pregnancy rates were similar between the two groups.
Conclusion(s): Cetrorelix and rFSH can be mixed together without compromising their reported safety and efficacy. This observation is in line with the reported safety and efficacy profile of the products listed in their current package inserts. (Fertil Steril® 2010;94:179–83. ©2010 by American Society for Reproductive Medicine.)

Key Words: Cetrorelix, FSH, premature LH surge, visual analogue scale

Ovarian stimulation is an important part of infertility treatment. Ovarian stimulation involves subcutaneous or intramuscular injections of various fertility drugs, including gonadotropins, GnRH agonist or antagonist, and hCG. Besides complications including multiple pregnancy and ovarian hyperstimulation syndrome (OHSS), injections of fertility drugs cause discomfort and psychologic stress, and require several hospital visits.

Mixing fertility drugs is appealing because it reduces the numbers of injections and hospital visits. Some physicians mixed FSH and LH (1) or FSH and human menopausal gonodotropins (hMG) (2, 3) in the same syringe and produced favorable outcomes. It has been shown that mixing FSH and hMG did not alter the expected bioactivity of either agent (2). Keye et al. (3) demonstrated that FSH and hMG could be mixed and produced adequate follicular growth, oocyte maturation, and excellent pregnancy rates. GnRH agonist (leuprolide acetate) and recombinant FHS (rFSH) can also be administered in a single injection with similar efficacy and patient tolerance (4, 5). Moreover, a study showed that ovarian stimulation using a single daily mixed injection combining GnRH agonist, rFSH alone, or in combination with rLH or hMG was efficient (6). The only report that mixed rFSH and GnRH antagonist (ganirelix) was by Klipstein et al. (7). Their results showed that mixing ganirelix and rFSH was safe and effective. However, the study was retrospective, and it did not compare the safety and efficacy of mixing ganirelix with rFSH versus separate injections of both products.

Therefore, we conducted a prospective, randomized study to assess the safety and efficacy of mixing GnRH antagonist and FSH. The purpose of the study was to evaluate if mixing cetrorelix and FSH would affect the pharmacologic activities of cetrorelix and/or FSH compared with separate injections. The primary efficacy endpoint was the incidence of

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Reprint requests: Jiann-Loung Hwang, M.D., Department of Obstetrics and Gynecology, Shin-Kong Wu Ho-Su Memorial Hospital, No. 95, Wen Chang Road, Shih Lin District, Taipei, Taiwan, Republic of China (FAX: +886-2-28389416; E-mail: M002179@ms.skh.org.tw).

premature LH surge. The secondary efficacy endpoints included estradiol (E_2) levels on the day of hCG injection, numbers of oocytes obtained, implantation rates, and ongoing pregnancy rates. The safety endpoints included adverse events related to ovarian stimulation (OHSS) and related to injections including local tolerability.

MATERIALS AND METHODS

The study was approved by the institutional review borad of the hospital. Each patient received detailed explanation of the study and signed the informed consent before the start of the study. The inclusion criteria were regular cyclic women of age <38 years, no history of ovarian surgery, day 3 FSH <12 mIU/mL, and a body mass index between 18.5 and 24.9 kg/m^2. The exclusion criteria include patients with polycystic ovarian syndrom (PCOS), severe endometriosis (American Fertility Society stages III and IV), or poor response in previous cycles (less than three oocytes retrieved), and patients who failed equal to or more than three IVF cycles. The patients were randomly allocated into one of the two groups using sealed envelopes and a random-number allocation table. The coordinator of the center assigned the patients to treatment protocols, and the physicians and the embryologists were blinded to the patients' treatment protocols. The incidence of premature LH surge was reported to be 1.56% (8). Assuming a mean difference should be <0.08 between the two groups, the sample size required would be 60, with each group to give a test of significance of 0.05 and a power of 0.8 (PS: power and sample size calculations, version 2.1.30).

Ovarian Stimulation

Ovarian stimulation consisted of subcutaneous injection of 225 IU recombinant FSH (rFSH, Gonal-f prefilled pen; Serono, Aubonne, Switzerland; 0.125 mL contains 75 IU) from cycle day 3. From day 5 of stimulation, the dose of rFSH was adjusted according to the follicular response, and 0.25 mg cetrorelix (Cetrotide; Serono, Frankfurt, Germany) was administered every day until the day of hCG injection. Cetrorelix powder was reconstituted with 1 mL of sterile water. In the mixed group, rFSH was injected into the vial containing cetrorelix solution. After mixing well, the solution was withdrawn with a 2.5-mL conventional syringe (Terumo, Binan, Philippines), with its needle replaced by a 30 gauge × 1/2″ needle (BD PrecisionGlide Needle; Becton-Dickinson, Franklin Lakes, NJ) for injection. In the separate group, a Gonal-f prefilled pen equipped with a 29 gauge × 1/2″ needle was used for injection. The cetrorelix solution was withdrawn with a 2.5-mL conventional syringe (Terumo, Binan, Philippines), also with the needle replaced by a 30 gauge × 1/2″ needle (BD PrecisionGlide Needle) before injection. The volumes of injections and the sizes of the needles used in the two groups were slightly different. A study nurse instructed the patients how to self-inject before ovarian stimulation. The injections were given subcutaneously in the abdominal wall around the umbilicus by the patients themselves between 1800 hours and 2000 hours, and the injection sites were rotated on a daily basis. Blood samples were taken for determination of E_2, LH, and progesterone between 0900 hours and 1100 hours. When at least three follicles had reached 17 mm, 250 μg hCG (Ovidrel; Serono, Bari, Italy) in vial was given. Oocyte retrieval was performed 36 hours later. Intracytoplasmic sperm injection (ICSI) was performed in all cycles to observe the morphology of the oocytes. Embryo transfer was performed on day 2 or day 3 after fertilization. A maximum of three embryos were transferred. Luteal support was by Crinone 8% gel (Fleet Laboratories, Watford, U.K.), 90 mg daily, from the day of oocyte retrieval until the day of pregnancy test, and in the case of pregnancy until week 10. Ongoing pregnancy was defined as a pregnancy progressing beyond 12 weeks of gestation. The physicians, ultrasonographers, and embryologists were blinded to the patients' injection protocols.

Local Tolerability

Tolerability to the administration was determined by use of a questionnaire given to patients at the start of ovarian stimulation. Every day at 5 minutes and 60 minutes after injection of the fertility drugs, the patients rated pain at the site of injection using a visual analogue scale (VAS) with scores from 0 (no pain) to 10 (extreme pain). Side effects including redness, swelling, bruising, and itching at the injection sites were assessed by the patients themselves 60 minutes after injection.

At the end of all injections, the patients were asked about their preference of administration. In the mixed group, the question was "If separate injections cause *less* pain, do you prefer separate injections or not?" In the separate group, the question was "If mixed injections cause *more* pain, do you prefer mixed injections or not?"

Hormonal Monitoring

On cycle day 3, every woman had a blood test for baseline FSH, LH, and E_2. From the day of cetrorelix injection until the day of Ovidrel injection, the subjects were checked for E_2, LH, and progesterone every day. LH was measured with immunometric assay using an Immulite kit (Diagnostic Products Corporation, Los Angeles, CA). The sensitivity and intra- and interassays coefficients of variation (CVs) were 0.1 mIU/mL, 6.5%, and 7.1%, respectively. E_2 and progesterone were measured by competitive immunoassay using an Immulite kit, with intra- and interassay CVs of 6.3% and 6.4% for E_2, and 6.3% and 5.8% for progesterone, respectively. Sensitivity was 15 pg/mL (55 pmol/L) for E_2 and 0.2 ng/mL (0.6 nmol/L) for progesterone.

An LH surge was defined as LH $\geq$ 10 mIU/mL and progesterone $\geq$ 1.0 ng/mL (9). A premature LH surge was defined as LH surge occurring before the administration of hCG.

Statistical Analysis

The results are expressed as mean ± standard deviation. Statistical analysis was performed by using SPSS software

TABLE 1

Patient characteristics.

Variable	Mixed group (n = 66)	Separate group (n = 66)	*P* value
Age (y)	33.4 ± 3.3	33.4 ± 2.9	NS
Duration of infertility (y)	3.3 ± 2.2	3.7 ± 2.0	NS
Body mass index (kg/m^2)	21.67 ± 2.77	20.95 ± 2.14	NS
Day 3 FSH (mIU/mL)	7.19 ± 1.80	7.49 ± 1.93	NS
Day 3 LH (mIU/mL)	4.08 ± 2.03	4.13 ± 1.61	NS
Day 3 E_2 (pg/mL)	34.39 ± 16.43	38.47 ± 15.98	NS

Note: Mean ± SD; NS = not significant.

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(Version 13.0; SSPS, Inc., Chicago, IL). Interval variables were compared by independent *t* test and categoric data were compared by chi-square test or Fisher's exact test. For the analysis of the pain score, Friedman's test was used for group comparison at each time point and repeated-measure analysis of variance with post hoc tests was used for the comparison at different time points in an individual group. Statistical significance was defined as $P<.05$.

RESULTS

From May 2006 to June 2007, 140 infertile women were enrolled into this prospective study. Seventy patients were allocated to the mixed (M) group and 70 patients to the separate (S) group. In the M group, four patients dropped from the study because of poor response, and shifted to IUI. In the S group, there were also four patients dropping from the study. One patient shifted to IUI because of poor response, one withdrew for personal reason, and two because of poor response. Therefore, there were 66 patients in each group for analysis. As shown in Table 1, no statistical differences were observed between the two groups about demographic and baseline hormone profiles.

As expected, the patients in the M group received significantly fewer injections than the patients in the separate group (9.1 vs. 13.9). Other efficacy parameters, including total numbers of oocytes retrieved, numbers of metaphase II oocytes, fertilization, implantation, and ongoing pregnancy rates were similar between the two groups (Table 2). All 66 patients in the S group received embryo transfer. Three out of 66 patients in the M group did not receive embryo transfer: one because no embryo was obtained, the other two had their embryos frozen because of fluid in endometrium or thin endometrium, respectively. Both of them became pregnant after thawed embryo transfer. There were 27 clinical pregnancies (41.0% per oocyte retrieval) in the mixed group and 23 clinical pregnancies (34.8% per oocyte retrieval) in the separate group.

The levels of E_2, LH, and progesterone on the day of cetrorelix injection and hCG injection were similar between the two groups. In fact, from the day of initiating cetrorelix

TABLE 2

Treatment characteristics.

Variable	Mixed group (n = 66)	Separate group (n = 66)	*P* value
rFSH used (IU)	2,014.0 ± 512.9	1,925.3 ± 425.8	NS
No. of injections[a]	9.1 ± 1.3	13.9 ± 1.9	.01
Days of cetrorelix injection	5.2 ± 1.3	5.0 ± 1.0	NS
Day of oocyte retrieval	12.2 ± 1.2	12.0 ± 1.0	NS
No. of oocytes retrieved	11.5 ± 4.9	10.9 ± 5.1	NS
No. of metaphase II oocytes	9.8 ± 4.0	9.8 ± 4.7	NS
Fertilization rate	78.30%	83.63%	NS
No. of embryos transferred	2.8 ± 1.1	2.9 ± 0.9	NS
Ongoing pregnancy (per started cycle)	27 (41.0%)	23 (34.8%)	NS
Implantation rate	27.6%	21.6%	NS

Note: Mean ± SD; NS = not significant.

[a]Including rFSH and cetrorelix.

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TABLE 3

Local tolerability at injection sites.

	Mixed group (n = 66)	Separate group (n = 66)	*P* value
Redness			
Stimulation day 1 to day 4	11 (16.7%)	13 (19.7%)	NS
After stimulation day 4	24 (36.4%)	38 (57.6%)	.015
Swelling			
Stimulation day 1 to day 4	7 (10.6%)	6 (9.1%)	NS
After stimulation day 4	21 (31.8%)	27 (39.4%)	NS
Bruising			
Stimulation day 1 to day 4	9 (13.6%)	7 (10.6%)	NS
After stimulation day 4	15 (22.7%)	23 (34.8%)	NS
Itching			
Stimulation day 1 to day 4	4 (6.1%)	10 (15.2%)	NS
After stimulation day 4	20 (30.3%)	32 (48.5%)	.033

Note: NS = not significant.

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until the day of hCG injection, daily E_2, LH, and progesterone levels were similar between the two groups (data not shown). There was no premature LH surge in the mixed group. One woman (1.5%) in the separate group developed premature LH surge on the second day of cetrorelix injection. LH level dropped on the next day, and she became pregnant. The incidences of premature LH surge in the two groups were not significantly different.

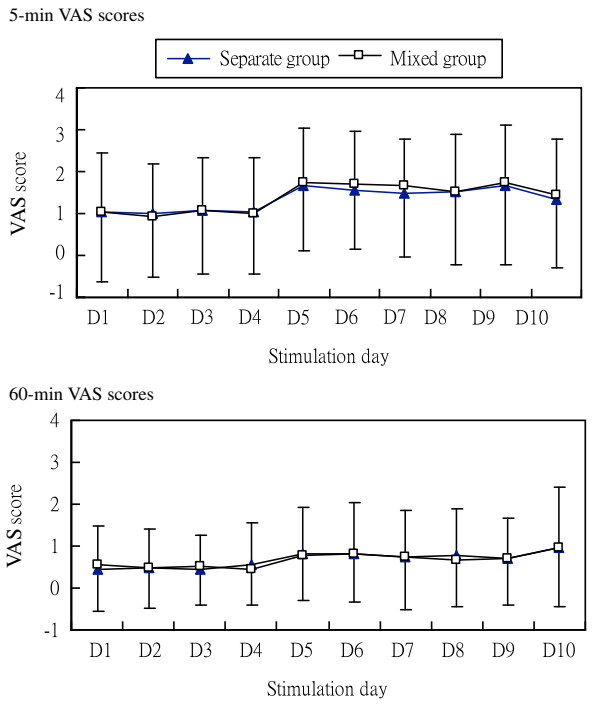
All patients in both groups returned the questionnaires. The incidences of adverse events from injections are shown in Table 3. In both groups, more patients experienced adverse events after cetrorelix was initiated. The adverse events in the first 4 days of stimulation were similar between the two groups. After the initiation of cetrorelix, the incidences of swelling and bruising were similar between the two groups, but more patients in the separate group experienced redness and itching.

The mean 5-minute VAS scores in the first 4 days and after 4 days in the mixed group were 1.0 (±1.4) and 1.4 (±1.4), respectively, and those of the separate group were 1.0 (±1.1) and 1.3 (±1.0), respectively. Sixty-minute VAS scores were lower than 5-minute scores in each group. The mean 60-minute VAS scores in the first 4 days and after 4 days in the mixed group were 0.5 (±0.9) and 0.6 (±0.9), respectively, and those of the separate group were 0.5 (±0.8) and 0.7 (±0.9), respectively. The daily VAS scores are shown in Figure 1. In both groups, 5-minute VAS scores increased after day 4, but were not statistically different. Sixty-mintue VAS scores were not statistically different every day. Every day, VAS scores of the two groups were similar.

When the patients in the mixed group were asked "If separate injections cause *less* pain, do you prefer separate injections or not?," seven patients (10.6%) answered "Yes," and 55 patients (83.3%) answered "No." The other four patients (6.1%) expressed "no comment." When the patients in the separate group were asked "If mixed injections cause *more* pain, do you prefer mixed injections or not?," 36 patients (54.5%) answered "Yes," and 23 patients (34.8%) answered "No," and the other six patients (9.0%) expressed "no comment." This suggests most of the patients prefer mixed injections, even if they might suffer more pain. The number of times the needle penetrates the skin seems to be very important to patients.

FIGURE 1

Five-minute and 60-minute VAS scores.



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DISCUSSION

This is the first prospective study to assess the safety and efficacy of mixing cetrorelix and rFSH in one injection. Although no pharmacokinetic study was performed, the study demonstrates that mixing cetrorelix and follitropin alfa is feasible and safe, and it is as efficacious as separate injections. The results, however, may not be extrapolated to the mixing of other fertility drugs.

The incidence of premature LH surge in our study was comparable to previous studies (8–11). The duration of cetrorelix injection and the incidences of premature LH surge were similar between the two groups, indicating the activity of cetrorelix was not affected by mixing rFSH and cetrorelix in the same syringe. No premature LH surge occurred in the M group.

Similarly, the outcomes of ICSI cycles and the amount of rFSH used showed no differences between the two groups, indicating the action of rFSH was not affected by mixing rFSH and cetrorelix.

Adverse events from injections were common, but they were mild. Mixing rFSH and cetrorelix did not increase the adverse events of injections. In both groups, more patients experienced adverse events after the initiation of cetrorelix. Unexpectedly, after initiating cetrorelix, the incidences of redness and itching were higher in the separate group. This is probably because more injections were given in the separate group or the addition of rFSH to cetrorelix changed some characteristics of the mixture compared with cetrorelix alone.

In both groups, most patients experienced low-grade injection-induced pain, and had good tolerability to either mixed or separate injections. Given in larger volume but thinner needle (30 vs. 29 gauge), mixed injections did not cause more pain. This is probably because we routinely used finer needles (30 gauge) instead of 22-gauge needles that go with the conventional syringes used with cetrorelix. Most of the patients preferred mixed injections when they have the chance to choose. When facing the suggestion in the questionnaire that mixed injections might cause more pain, more patients in the separate group still preferred mixed injections. This reflects the inherent fear of needle injection in humans. Similarly, most patients in the mixed group insisted on their preference for mixed injections because they felt the pain associated with mixed injections was acceptably mild. Accordingly, fewer injections by mixing the fertility drugs together may be a better injection protocol regarding physical and psychologic acceptability, as long as more evidence proves that the mixture produces no inferior efficacy or chemical interaction.

Although there might be a concern that mixing fertility drugs complicates ovarian stimulation, our patients were not bothered by the preparation of fertility drug mixture. In fact, there were no any phone calls inquiring about the preparation of injections, nor were there any incorrect injections.

A patient-friendly protocol may encourage patients to undergo more assisted reproductive technology (ART) treatments. Although mild stimulation has been proposed to result in less discomfort, fewer hospital visits, reduced cost, and lower risks of OHSS, and multiple pregnancies, it also involves higher cancellation rate (12). Compared with mild stimulation, mixed injection is patient-friendly without compromising the outcome of ART.

In conclusion, this study shows that cetrorelix and rFSH can be mixed together without compromising products' safety and efficacy. Mixed injection not only simplifies ovarian stimulation but also increases patient acceptance. Higher acceptance may encourage patients to undergo more ART cycles.

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