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

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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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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