The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study

Juan A. Garcia-Velasco, M.D., Luis Moreno, M.D., Alberto Pacheco, Ph.D., Alfredo Guillén, M.D., Luis Duque, M.D., Antonio Requena, M.D., and Antonio Pellicer, M.D.*

IVI-Madrid, Rey Juan Carlos University, Madrid, Spain

Objective: To evaluate the impact of aromatase inhibitors as adjuvant treatment in IVF cycles on intraovarian androgens and cycle outcome.
Design: Observational, pilot study.
Setting: University-affiliated IVF unit.
Patient(s): One hundred forty-seven low responder patients with a previous canceled IVF cycle; 71 patients were treated with letrozole 2.5 mg plus a high-dose FSH/hMG-antagonist regimen, and 76 patients were similarly treated but letrozole was not employed.
Intervention(s): In vitro fertilization treatment with an antagonist FSH/hMG protocol with or without letrozole was administered during the first 5 days of stimulation; hormones were evaluated in both serum and follicular fluid.
Main Outcome Measure(s): Number of oocytes retrieved, fertilization rate, implantation rate, and pregnancy rate; androstenedione, T, E_2, and P values in serum and follicular fluid.
Result(s): Letrozole-treated patients showed significantly higher levels of follicular fluid T and androstenedione (80.3 vs. 43.8 pg/mL and 57.9 vs. 37.4 mg/mL, respectively). Similarly, these patients had a higher number of oocytes retrieved (6.1 vs. 4.3) and a higher implantation rate (25% vs. 9.4%) despite similar doses of FSH/hMG (3,627 vs. 3,804 IU).
Conclusion(s): Adding 2.5 mg of letrozole to a high-dose FSH/hMG antagonist protocol increases intraovarian androstenedione and T concentration and improves IVF cycle outcome in poor responder patients. (Fertil Steril® 2005;84:82–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Poor response, IVF, aromatase inhibitors

Low ovarian response to controlled ovarian hyperstimulation (COH) is still a major concern in assisted reproduction. Poor response to gonadotropins is strongly related to diminished ovarian reserve and advanced maternal age, as both have a direct and profound impact on the success of assisted reproductive technologies. It has been shown that diminished ovarian reserve affects mainly egg production rather than egg quality, a characteristic strongly influenced by maternal age (1, 2).

Among the different predictors of poor ovarian response in IVF cycles, age, basal follicle-stimulating hormone (FSH), and antral follicle count seem to perform the best (3, 4). However, some young patients with normal FSH concentrations present repeated low responses to aggressive stimulation protocols. Obviously, ovarian response to stimulation is the ultimate test (5).

In these young patients with either elevated basal FSH or low antral follicle count or a previous cycle with a low response, different strategies have been assayed to improve their outcome. The majority of these strategies aim to recruit a higher number of follicles either by increasing the dose of gonadotropins, by reducing the dose of GnRH analogs, or even by optimizing the endogenous FSH flare effect (6–8). Even though it may increase ovarian response, implantation rates remain the same (9, 10).

A lower expression of FSH receptor in the granulosa cells from low responder patients has been demonstrated (11). Contrary to this situation are patients with polycystic ovaries (PCOs), who show a hyperexpression of FSH receptor in their follicular granulosa cell population, probably the reason

Received September 16, 2004; revised and accepted January 24. 2005.
Reprint requests: Juan A Garcia-Velasco, M.D., IVI-Madrid, Santiago de Compostela 88, 28035 Madrid, Spain (FAX: 91-386-7133; E-mail: jgvelasco@ivi.es).

0015-0282/05/$30.00
doi:10.1016/j.fertnstert.2005.01.117

why they show a tendency to hyperrespond to COH. As these PCO patients show high serum and follicular fluid LH and androgens concentrations (12), we might speculate that inducing a temporary and reversible PCO-like condition in the ovaries of poor responder patients could enhance their follicular recruitment and development.

Letrozole is a potent and highly specific nonsteroidal aromatase inhibitor that initially was approved for use in postmenopausal women with breast cancer to suppress estrogen production (13). It inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme resulting in a blockade of androgens conversion into estrogens with a subsequent increase in intraovarian androgens (14). Letrozole, at doses of 1–5 mg/day, inhibits aromatase activity by 97%–99% (15).

A recent study has shown that androgens, in addition to serving as precursors for ovarian estrogen synthesis, also have a fundamental trophic role in primate ovarian follicular development by augmentation of FSH receptor expression on granulosa cells (16). This mechanism may explain the frequent hyperresponse to ovarian stimulation of PCO patients. In fact, previous work has shown that aromatase inhibition improves ovarian response to FSH in poor responder patients undergoing ovulation induction and intrauterine insemination (17). Only limited, retrospective data are available in IVF/intracytoplasmic sperm injection cycles, suggesting that empirically adding an aromatase inhibitor may benefit patients who failed a previous cycle (18, 19). However, no evidence is provided to justify their results.

The specific aims of this prospective, pilot study were [1] to assess whether aromatase inhibition with letrozole improves ovarian response and IVF cycle outcome in low responder patients undergoing IVF and [2] to quantify serum and follicular fluid concentration of E_2, T, and androstenedione in both groups to evaluate the effect of letrozole.

MATERIALS AND METHODS

Study Design

This study included a total of 147 IVF cycles performed in 147 low responders treated at our institution between November 1, 2002, and February 28, 2004. The study was approved by our institution's ethics committee, and all couples were required to sign a written informed consent after the provision of complete information.

To be included in this study, patients had to have at least one previous canceled IVF attempt in which four or fewer follicles 16 mm in diameter were obtained and/or serum E_2 levels were $\leq$500 pg/mL and basal FSH concentrations were $<$12 IU/mL (8). There were no exclusion criteria and there was no age limit. The canceled cycle was stimulated with a long protocol combined with high doses of FSH/hMG, as described elsewhere (6). Briefly, after pituitary desensitization with a GnRH agonist (triptorelin; Decapeptyl 0.1 mg, Ipsen Pharma, Barcelona, Spain), basal vaginal ultrasound was performed to ascertain ovarian quiescence on the first 3 days of menses. Then ovarian stimulation was started with recombinant FSH (Gonal F, Serono, Madrid, Spain) 225 IU/day together with 150 IU of highly purified hMG (Menopur, Ferring S.A., Madrid, Spain) for the first 3 days, and then individual dose adjustments were done as required, according to serum E_2 concentrations and ovarian response (mean gonadotropin dose in the previous cycle, 3,400 $\pm$ 181 IU).

During their next cycle, the patients were informed about the possibility of being included in the study by adding or not adding letrozole 2.5 mg to the first 5 days of ovarian stimulation. Seventy-one patients were treated in 71 cycles with a high-dose regimen and letrozole, and 76 were treated in 76 cycles with a high-dose regimen alone. Successive ovarian stimulation was separated by a minimum of 2 or more months to avoid any potential source of error, as this is our routine clinical practice.

Patients and Stimulation Protocol

The etiologies of infertility were 41% male factor, 12% tubal disease, 15% unexplained, and 32% a combination of male and female factors. No patient had any uterine anomaly. The etiology of infertility was equally distributed between groups. All patients had regular menstrual cycles every 26–32 days and were not taking any medication. The mean ($\pm$SEM) age of the patients included was 37.1 $\pm$ 0.5 years, and the body mass index was 22.9 $\pm$ 0.5 kg/m^2, with no differences between groups. The mean duration of infertility was 3.5 $\pm$ 0.7 years (range, 1–6 years).

Stimulation Protocols

The protocol for ovarian stimulation was initiated with the administration of an oral contraceptive pill the month before the cycle (0.05 mg ethinylestradiol + 25 mg levonorgestrel, Neogynona, Schering AG, Berlin, Germany). Serum E_2 concentrations $<$60 pg/mL (220 pmol/L) and negative findings (absence of ovarian cysts $>$10 mm diameter) on vaginal ultrasound scans performed on cycle day 1 or 2 were used to define ovarian quiescence. If a cyst $>$10 mm diameter was observed, then a serum E_2 concentration $<$60 pg/mL was sufficient to confirm ovarian quiescence. If serum E_2 concentrations were beyond the cut-off point, the patient was excluded from the study.

Briefly, on days 1–4 of ovarian stimulation, 225 IU of recombinant FSH (rFSH) (Gonal-F 75; Serono) was administered together with 150 IU of highly purified hMG (Menopur, Ferring). Beginning on day 5, rFSH/hMG was administered on an individual basis according to serum E_2 concentrations and transvaginal ovarian ultrasound scans. For the first 5 days of stimulation, 71 patients were additionally treated with letrozole (Femara, Novartis, Barcelona, Spain) 2.5 mg/day.

Starting when leading follicles reached 14 mm in mean diameter, 0.25 mg of the GnRH antagonist ganirelix (Or-