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Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders

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Objective: To examine the use of the aromatase inhibitor letrozole with FSH for ovarian stimulation in poor responders undergoing ovarian superovulation and IUI.

Design: Observational cohort study as a prospective clinical trial in patients with unexplained infertility and a low response to ovarian stimulation with FSH.

Setting: Two tertiary referral infertility clinics associated with the Reproductive Sciences Division, University of Toronto.

Patient(s): Twelve patients with unexplained infertility undergoing IUI who received FSH alone in 25 prior cycles with poor response (less than three dominant follicles).

Intervention(s): Patients were offered letrozole, 2.5 mg/day from day 3–7 of the menstrual cycle with FSH (50–225 IU/day) starting on day 5–7. hCG (10,000 IU) was given when two leading follicles were ≥2 cm followed by IUI.

Main Outcome Measure(s): Number of mature follicles (>1.8 cm), FSH dose, endometrial thickness, and pregnancy rate.

Result(s): Improved response to FSH stimulation with letrozole co-treatment was evidenced by the significantly lower FSH dose associated with significantly higher number of mature follicles. During letrozole plus FSH stimulation cycles, clinical pregnancy was achieved in three cycles (21%).

Conclusion(s): In this preliminary report, we demonstrate a potential benefit of aromatase inhibition for improving ovarian response to FSH in poor responders. (Fertil Steril® 2002;77:776–80. ©2002 by American Society for Reproductive Medicine.)

Key Words: Aromatase, letrozole, aromatase inhibitors, FSH, ovarian stimulation, low responders, intrauterine insemination

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Ovarian superovulation with gonadotropin injection and IUI are used alone or in combination for the management of unexplained infertility, male factor infertility, and other cases of infertility in which the female partner has open fallopian tubes and ovarian function and the male partner has motile sperm (1).

Achievement of multiple follicle development during gonadotropin stimulation has been found to correlate positively with the outcome of infertility management in terms of achievement of pregnancy. Although there is a lack of standard definitions, low responders may be considered to be patients who fail to achieve a target number of mature follicles or adequate E_2 levels after gonadotropin stimulation. A low ovarian response to FSH stimulation occurs in about 10%–25% of patients undergoing controlled ovarian hyperstimulation, and still represents one of the most intractable problems of infertility treatment (2, 3). There is a general agreement that low responders have a lower pregnancy rate than their normally responding counterparts.

Low ovarian response may be a result of diminished ovarian reserve, which can be due to advanced age, prior ovarian surgery, and environmental and genetic factors (4). Also other factors such as severe endometriosis (5) and pelvic infections (6) may impair ovarian function. However, in most patients, low ovarian response to FSH stimulation remains unexplained (7).

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TABLE 1

Patients' characteristics.

Characteristics	Mean + SD	Median	Range
Age (years)	35.5 ± 5.07	38	24–41
Duration of infertility (years)	3.5 ± 1.431	3	2–7
Day 3 FSH (IU/L)	7.1 ± 1.6	7	5–10.65

Mitwally. Aromatase inhibition for low responders. Fertil Steril 2002.

ovaries including day 3 FSH, clomiphene citrate (CC) challenge test, and inhibin concentrations can be used to predict low response to ovarian stimulation. However, there still remain patients who respond poorly to stimulation in spite of having normal results of these ovarian reserve tests. Low ovarian response may, therefore, not be predictable until a patient has demonstrated inadequate ovarian stimulation with a standard stimulation protocol (8). Despite a number of developments in ovarian stimulation protocols, low response to ovarian stimulation remains a problem and the attempts to improve ovarian response to gonadotropin stimulation have shown variable success.

Aromatase is a cytochrome P-450 enzyme complex that catalyzes the rate-limiting step in the production of estrogens (E), that is, the conversion of the androgens, androstenedione and testosterone, to estrone (E_1) and E_2 (9). This enzyme is a good target for selective inhibition because E production is a terminal step in the hormonal biosynthetic sequence. The lack of specificity and unfavorable toxicity profile of the first generation aromatase inhibitors, such as aminoglutethimide, have led to the development of more specific and potent aromatase inhibitors, including letrozole. Letrozole is orally active with in vitro and in vivo potency of up to 1,000 times that of aminoglutethimide. It has been approved clinically for E suppression in postmenopausal patients with breast cancer. Treatment in this group of patients was tolerated well with no significant side effects (10).

We have reported the success of aromatase inhibition by letrozole in inducing ovulation in anovulatory women with polycystic ovary syndrome (PCOS) (11–14) and augmenting ovulation in ovulatory women (13, 14). Moreover, we have shown that when letrozole was used with FSH, a significant reduction occurred in FSH dose needed for controlled ovarian hyperstimulation (15, 16).

We believe that blockade of E synthesis by an aromatase inhibitor in the early part of the menstrual cycle will decrease E negative feedback centrally, resulting in increased gonadotropin secretion. Moreover, by blocking the conversion of androgens to E in the ovary, the accumulating intraovarian androgens may increase follicular sensitivity through amplification of FSH receptor gene expression (17–20).

The objective of this pilot study was to examine whether the use of letrozole with FSH could improve ovarian response in poor responders undergoing ovarian superovulation and IUI.

PATIENTS AND METHODS

Approval by the Research Ethics Board of The University of Toronto and Mount Sinai Hospital was obtained for the use of the aromatase inhibitor letrozole for ovarian stimulation. The study was conducted in the Reproductive Biology Unit, Toronto General Hospital, Mount Sinai Hospital, and the Toronto Center for Advanced Reproductive Technology; these clinics are affiliated with the Reproductive Sciences Division, Department of Obstetrics and Gynecology, University of Toronto, Canada. Patients were enrolled in the study between January 2000 and March 2001.

This was an observational cohort study conducted as a prospective clinical trial that included 12 patients with unexplained infertility with a low response to ovarian stimulation with FSH in at least two cycles (total of 25 cycles of FSH-only stimulation). Poor response was defined as less than three follicles >1.8 cm in diameter on the day of LH surge or hCG administration. Patients were offered letrozole as an adjuvant treatment with FSH injection to improve ovarian response to FSH stimulation after being counseled regarding the nature of letrozole as an aromatase inhibitor being used experimentally for a new indication. Nine patients had primary infertility and three had secondary infertility. The age and duration of infertility are shown in Table 1 in addition to day 3 FSH levels that were measured before enrollment into the program.

During the prior FSH stimulation (without letrozole), highly purified FSH (Fertinorm, Serono, Oakville, Ontario, Canada) or recombinant FSH (Puregon, Organon, Scarborough, Ontario, Canada or Gonal-F Serono, Oakville, Ontario, Canada) were given at a dose of 75–300 IU/day starting usually on day 3 of the menstrual cycle. Highly purified FSH was given in 11 cycles and recombinant FSH was given in 14 cycles.

During letrozole plus FSH stimulation cycles, letrozole (Femara; Novartis, East Hanover, NJ) was given at a dose of 2.5 mg from day 3 to 7 of the menstrual cycle and FSH

TABLE 2

Comparison of the different cycle parameters between the completed FSH-only stimulation cycles (20 cycles) and the FSH plus letrozole stimulation cycles (14 cycles).

Variables	FSH only (20 cycles)	Letrozole + FSH (14 cycles)	*P* Value
Total FSH/cycle (IU)	1590 ± 708	616 ± 454	.0001
Stimulation days/cycle	9 ± 3.42	6.57 ± 1.95	.026
Day of hCG administration	11.6 ± 2.27	11.78 ± 0.939	NS
No. of mature follicles (>1.8 cm)	1.9 ± 0.6	3.3 ± 1.8	.003
E2 on hCG day (pmol/L)	2471 ± 1113	1786 ± 898	NS
E2/mature follicle on hCG day (pmol/L)	1324 ± 485	626 ± 302	.0001
Endometrial thickness on hCG day (cm)	0.89 ± 0.1	0.88 ± 0.09	NS
LH on hCG day (IU/L)	10.3 ± 4.3	16.7 ± 13.7	NS

Note: Values are expressed as mean ± SD.
NS = Not significant.

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injection started usually on day 7 of the menstrual cycle at a dose of 50–225 IU/day. Highly purified FSH was used in two cycles and recombinant FSH was used in 12 cycles.

During the FSH-only and letrozole plus FSH stimulation cycles, the primary treating physician decided the initial starting dose of FSH injection depending on the clinical profile of the patient. The FSH dose was adjusted during follicular monitoring in an attempt to obtain a total of three mature follicles (>1.8 cm).

Ovarian follicular development was monitored by transvaginal ultrasonography and serum levels of E_2 and LH. Subcutaneous hCG 10,000 IU (Profasi, Serono, Oakville, Ontario, Canada) was given to trigger ovulation when two leading follicles reached a diameter >2 cm. The hCG administration was followed by IUI on the following day if spontaneous LH surge occurred on the day of hCG, or 2 days after hCG administration if no spontaneous LH surge occurred. Pregnancy was diagnosed by quantitative assay of serum β-hCG 2 weeks after hCG injection and clinical pregnancy was confirmed by identification of a positive fetal heart beat at 7–8 weeks' gestation by transvaginal ultrasonography.

Statistics

The various outcome measures were compared between the 20 FSH-only cycles (excluding the canceled cycles) and 14 cycles in which letrozole was administered with FSH injection. A group *t*-test or Student's *t*-test was used to compare data. The statistical tests were performed with GraphPad Prism Version 3 software (GraphPad Software Inc., San Diego, CA).

RESULTS

Twelve patients with unexplained infertility received FSH alone in 25 stimulation cycles; each patient had two cycles and one patient had three cycles of treatment. Five cycles were discontinued because of inadequate response (FSH injection was stopped around day 9 of the cycle and follicular monitoring discontinued when only one follicle was found to be growing). After having inadequate response during the FSH alone stimulation cycles, patients completed one (n = 12) or two (n = 2) stimulation cycles with FSH plus letrozole (n = 14 cycles) (Table 2).

There was no pregnancy during the FSH alone stimulation cycles, whereas four patients achieved pregnancy during letrozole plus FSH treatment cycles. Three had clinical pregnancies (21%) and one had a chemical pregnancy.

During FSH plus letrozole stimulation cycles, the mean number of mature follicles was 3.3, which was significantly higher than in the FSH-only stimulation cycles (1.9 follicles). The amount of FSH required was significantly lower in the letrozole plus FSH cycles than the FSH-only cycles.

There was no significant difference between FSH-only or letrozole plus FSH on the day of hCG administration, LH level, or endometrial thickness on the day of hCG administration indicating the absence of any antiestrogenic effects of letrozole on the endometrium. Although there was no difference in the level of E_2 on the day of hCG administration, the amount of E per mature follicle was significantly lower with letrozole treatment.

DISCUSSION

In this observational cohort clinical trial, we demonstrate a potential benefit of aromatase inhibition by letrozole in improving ovarian response to FSH stimulation in poor responders. The improved response is clearly shown by the significantly higher number of mature follicles and significantly lower amount of FSH (total amount per cycle and number of stimulation days) needed to achieve such im-

proved response with letrozole. In addition, three clinical pregnancies were achieved in the letrozole and FSH cycles.

Several strategies have been proposed to improve outcome in low responders including increasing the dose of FSH (21), using co-treatment of FSH and CC (22, 23), E (24), growth hormone (25) or birth control pills (26), and using GnRH agonist in different protocols (27). Unfortunately, all of these strategies have met with only limited success and no single strategy has proved to be significantly superior to the others. What makes the problem of poor responders even worse is that patients who fail to respond to repeated ovarian stimulation with FSH may have a higher chance of developing ovarian failure within a short time span (28).

After our first reports of the success of aromatase inhibition with letrozole in inducing and augmenting ovulation without the antiestrogenic drawbacks of CC, we wanted to explore its potential benefit for poor responders to ovarian stimulation. We believe that letrozole may act through different mechanisms, both centrally and peripherally. Centrally, aromatase inhibition, by reducing E production to postmenopausal levels in the hypothalamus and pituitary, may enhance gonadotropin secretion by releasing negative feedback of E. We have preliminary data to support this mechanism of action in the form of increased FSH and LH during letrozole treatment (data not shown). This central action likely mimics the mechanism of action of CC, but with the major advantage of no E receptor depletion as seen with an E antagonist.

Peripherally, letrozole may increase follicular sensitivity to FSH as a result of a temporary accumulation of intraovarian androgens, as the conversion of the androgen substrates to E is blocked. Recent data support a stimulatory role for androgens in early follicular growth in primates (17). Testosterone was found to augment follicular FSH receptor expression in primates suggesting that follicular growth may be improved by amplification of FSH effects (18–20). In addition, androgen accumulation in the follicle may stimulate insulin-like growth factor I (IGF-I), and other endocrine and paracrine factors, which may synergize with FSH to promote folliculogenesis (29–31). Therefore, a possible peripheral mechanism through the IGF system may be similar to that proposed for growth hormone in improving response to ovarian stimulation in poor responders.

Although entirely speculative at present, a peripheral mechanism of action for aromatase inhibition is exciting because it suggests that the use of aromatase inhibitors could also result in improved follicular sensitivity to FSH stimulation during IVF cycles in which GnRH agonist down-regulation abolishes the possibility of a central increase in gonadotropin secretion.

Letrozole treatment was associated with a significantly lower amount of E per mature follicle but still within physiologic levels seen during natural cycles. Lowering E_2 in COH cycles may be beneficial in avoiding the possible drawbacks of the supraphysiologic levels of E associated with ovarian superovulation. Markedly elevated E levels have been reported to have deleterious effects on the embryo or the endometrium and jeopardize the chance of achieving pregnancy (32, 33).

This preliminary clinical report includes a small number of patients treated in a nonrandomized, nonplacebo controlled design. However, our objective was to explore the idea of applying aromatase inhibition to improve ovarian response in a selected group of patients who had repeated poor response in prior FSH stimulation cycles. The results of this study are encouraging and can be used to provide the preliminary data needed for power calculations for sample size in a controlled prospective randomized trial in the future.

Because letrozole and other new aromatase inhibitors have not been extensively used in women of reproductive age, we do not know the possible effects of a short course of these medications for ovarian follicular stimulation on pregnancy outcome. However, the relatively brief half-life of letrozole (45 hours) and other nonsteroidal aromatase inhibitors should allow the rapid disappearance of the drug long before the critical period of embryogenesis, more likely even before implantation. It is important to address this issue clearly when potential infertility patient users are being counseled and to carefully follow up any resulting pregnancies.

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