

## BRIEF REPORT

# Relative Potencies of Anastrozole and Letrozole to Suppress Estradiol in Breast Cancer Patients Undergoing Ovarian Stimulation before *in Vitro* Fertilization

Amr A. Azim, Maria Costantini-Ferrando, K. Lostritto, and Kutluk Oktay

Department of Obstetrics and Gynecology, Weill Medical College of Cornell University, New York, New York 10021

**Context:** Breast cancer patients undergoing controlled ovarian hyperstimulation (COH) for embryo or oocyte cryopreservation should be induced by the method that leads to the least increase in estradiol ( $E_2$ ) levels.

**Objective:** The aim of the study was to determine the potency of anastrozole to suppress serum  $E_2$  levels in breast cancer patients undergoing COH.

**Design and Setting:** A prospective sequential cohort study was conducted in an academic center for reproductive medicine between May 2003 and November 2005 for letrozole and between December 2005 and April 2006 for anastrozole.

**Patients:** Breast cancer patients presenting for fertility preservation participated in the study.

**Intervention:** COH using FSH and letrozole ( $n = 47$ ) or anastrozole ( $n = 7$ ) was followed by oocyte retrieval and embryo cryopreservation.

**Main Outcome Measures:** Serum  $E_2$  levels, area under the curve for  $E_2$ , and outcomes of COH cycles were measured.

**Results:** There were no significant differences between the two groups regarding length of stimulation, total gonadotropin dose, number of follicles larger than 17 mm, and the lead follicle size on human chorionic gonadotropin (hCG) day and number of embryos cryopreserved. The mean  $E_2$  levels on the day of hCG and post-hCG days were higher in the anastrozole group compared to the letrozole group ( $1325.89 \pm 833.17$  and  $2515.07 \pm 1368.52$  vs.  $427.78 \pm 278.24$  and  $714.38 \pm 440.83$  pg/d/ml;  $P \leq 0.01$ ), respectively, even when anastrozole dose was increased up to 10 mg/d. The mean area under the curve was significantly higher in the anastrozole group compared to the letrozole group ( $4402.93 \pm 1526.7$  vs.  $1287.48 \pm 732.17$  pg·d/ml;  $P < 0.004$ ).

**Conclusions:** Breast cancer patients who underwent ovarian stimulation with anastrozole had a significantly higher exposure to  $E_2$  than those who were stimulated with letrozole. (*J Clin Endocrinol Metab* 92: 2197–2200, 2007)

A CONSIDERABLE BODY of data on the use of aromatase inhibitors for ovulation induction has accumulated in recent years (1). Third-generation aromatase inhibitors (letrozole, anastrozole, and exemestane) entered practice primarily as first and second line treatment agents for the treatment of breast cancer (2, 3). The use of aromatase inhibitors for ovulation induction was first reported in 2001, where letrozole provided superior results to clomiphene and was associated with 50% lower estradiol ( $E_2$ ) levels (4).

$E_2$  rise during controlled ovarian hyperstimulation (COH) may not be safe in women diagnosed with breast cancer seeking fertility preservation. It has been clearly shown that estrogen stimulates breast cancer cell growth, even in low concentrations (5, 6). Using the ability of letrozole to suppress  $E_2$  levels during ovarian stimulation, we recently used this drug in combination with FSH for ovulation induction to cryopreserve embryos or oocytes in breast cancer patients

before chemotherapy. The combined letrozole-FSH protocol resulted in peak  $E_2$  levels close to those seen in unstimulated cycles, and breast cancer recurrence rates were not increased compared with controls (7).

Anastrozole is a nonsteroidal, competitive inhibitor of the aromatase enzyme, thus blocking the conversion of testosterone and androstenedione to  $E_2$  and estrone in ovarian and peripheral tissues. Anastrozole is generally considered to be 2.5 times more potent in suppressing estrogen levels than letrozole (8). It has been shown that 1 mg/d anastrozole can suppress *in vivo* aromatization by 70% after 24 h, and by 80% after 14 d of administration (9). There have been a few reports on the use of anastrozole for ovulation induction (10, 11), but there are no reports on the use of anastrozole in the setting of COH before *in vitro* fertilization.

Recently, questions were raised regarding the safety of using letrozole in women (12) who are attempting to conceive, although this was not substantiated in later studies (13). We began our comparative study amid that controversy to develop an alternative to letrozole for ovarian stimulation in breast cancer patients. The specific aims of this study were to determine the feasibility of controlled ovarian stimulation in breast cancer patients using anastrozole and to determine

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Abbreviations: AUC, Area under the curve; COH, controlled ovarian hyperstimulation;  $E_2$ , estradiol; hCG, human chorionic gonadotropin.

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its potency in estrogen suppression relative to letrozole in reproductive age women.

### Patients and Methods

This was a prospective sequential cohort study of breast cancer patients (stages I to IIIa) who were referred between June 2003 and April 2006 for fertility preservation. The study was approved by the Institutional Review Board of New York Presbyterian Hospital. Informed consent was obtained from all patients. The inclusion criteria were: age 18 to 45 yr, histologically confirmed invasive breast cancer, no prior chemotherapy, no prior oophorectomy, regular menstrual cycles, as well as normal basal (d 2 or 3) FSH less than 13 mIU/ml and  $E_2$  less than 75 pg/ml (275.3 pmol/liter; conversion factor, 3.671). The approval of the patients' oncologist was obtained before the initiation of ovarian stimulation. Patients were enrolled to letrozole + FSH protocol between May 2003 and November 2005, and to anastrozole + FSH protocol between December 2005 and April 2006. The study was terminated when it was determined that anastrozole did not suppress  $E_2$  levels at the maximum tolerated dose (10 mg). Data for the letrozole + FSH protocol were drawn from the population of patients reported in a recent publication (7).

### Ovarian stimulation

Ovarian stimulation was performed using either letrozole (Femara, 5 mg/d; Novartis, East Hanover, NJ), as previously described (7, 14–15), or anastrozole (Arimidex, 2–10 mg/d; AstraZeneca, Wilmington, DE) in combination with gonadotropins. Aromatase inhibitors were started on the second day of the menstrual cycle and continued until the day of human chorionic gonadotropin (hCG). Letrozole dose was kept steady at 5 mg/d because previous work showed that  $E_2$  levels are sufficiently suppressed at that dose in women undergoing COH (7, 14). Anastrozole was begun at a dose of 2 mg/d, and the dose was increased to 10 mg/d as needed to suppress  $E_2$  levels. Specifically, if  $E_2$  levels had increased by more than 20% per day, the dose was increased by 1–2 mg. If hCG criteria were not obtained and  $E_2$  levels exceeded 400 pg/ml, the next patient was begun at the next higher dose. In patients receiving 6 mg or more per day of anastrozole, the dose was divided between the morning and bedtime (Fig. 1).

To prevent a premature LH surge, a GnRH antagonist (Ganirelix, 250  $\mu$ g/d; Organon, West Orange, NJ) was administered when  $E_2$  was at least 250 pg/ml (917.75 pmol/liter) or the lead follicle size reached 14 mm in mean diameter.

Daily injections of recombinant FSH (Follistim, Organon; or Gonal-F, Serono, Rockville, MD) with or without human menopausal gonadotropins (Repronex, Ferring, Tarrytown, NY) were added 2 d after the initiation of COH (7, 14). The starting dose of FSH ranged between 150 and 300 U, and of human menopausal gonadotropin between 0 and 150 U. hCG was administered when at least two follicles reached at least 19 mm diameter (7). Transvaginal oocyte retrieval was performed approximately 36 h after hCG administration. All oocytes were fertilized by

intracytoplasmic sperm injection. Embryos were cryopreserved by slow freezing at the prezygote (2-pronuclei) stage in all cycles in both groups.

### Hormone analysis

FSH and LH were measured using a solid-phase chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA). The FSH assay has a sensitivity of 0.1 mIU/ml, and the LH assay has a sensitivity of 0.05 mIU/ml.  $E_2$  was quantified using in-house RIA (Direct  $^{125}$ I, Pantex, Santa Monica, CA). The assay has a minimum sensitivity of 10 pg/ml, an intraassay coefficient of variation of 4.2–16%, and an interassay coefficient of variation of 7.3–15.5%.

### Statistical analysis

Demographic characteristics, hormonal data, and *in vitro* fertilization outcomes were presented as mean  $\pm$  SD. Student's *t* test or one-way ANOVA was used for comparison of population means.  $P < 0.05$  was considered statistically significant. SPSS version 11.3 (SPSS, Inc., Chicago, IL) was used for statistical analysis. Curve fitting for mean  $E_2$  values was performed using Prism 4 software (GraphPad Inc., San Diego, CA). Mean area under the curve (AUC) for  $E_2$  levels was performed using NCSS software (release December, 2006; NCSS, Kaysville, UT).

## Results

### Dose

The mean anastrozole dose was 5.7 mg/d, nearly six times the dose used for breast cancer, whereas mean letrozole dose was 5 mg/d, twice the dose used in breast cancer. The maximum doses of anastrozole that were used were 10 mg (one patient), 8 mg (one patient), 6 mg (one patient), 5 mg (one patient), 4 mg (two patients), and 2 mg (one patient).

### Adverse events

Aromatase inhibitors were well tolerated during stimulation in the majority of patients. One patient in the letrozole group developed phlebitis of tarsal veins, and the cycle was cancelled. In the anastrozole group, two patients experienced gastric irritation at 8- and 10-mg doses. The symptoms were severe enough at 10 mg to preclude further dose increases.

### Ovarian stimulation outcome

Results of COH in the study groups are presented in Table 1. There were no significant differences between the two groups regarding age, d-2 FSH, LH,  $E_2$ , length of stimulation, total gonadotropin dose, number of follicles larger than 17 mm, and the lead follicle size on hCG day.

The mean  $E_2$  on the day of hCG was  $1325.89 \pm 833.17$  pg/ml for anastrozole and  $427.78 \pm 278.24$  pg/ml for letrozole ( $P < 0.01$ ). On post-hCG day,  $E_2$  was  $2515.07 \pm 1368.52$  for anastrozole vs.  $714.38 \pm 440.83$  pg/ml for letrozole ( $P = 0.01$ ). Overall, mean  $E_2$  levels in the anastrozole group were significantly higher than letrozole after the fifth day of COH until hCG administration (Fig. 2). Even at the 10-mg dose, anastrozole failed to suppress  $E_2$  levels (2322.4 pg/ml or 8525.5 pmol/liter on day of hCG).

To quantify and compare the total  $E_2$  exposure between the two groups, area under the curve (AUC) analysis was performed. The mean AUC was significantly higher in the anastrozole group compared with the letrozole group ( $4402.93 \pm 1526.7$  vs.  $1287.48 \pm 732.17$  pg·d/ml;  $P < 0.004$ ).

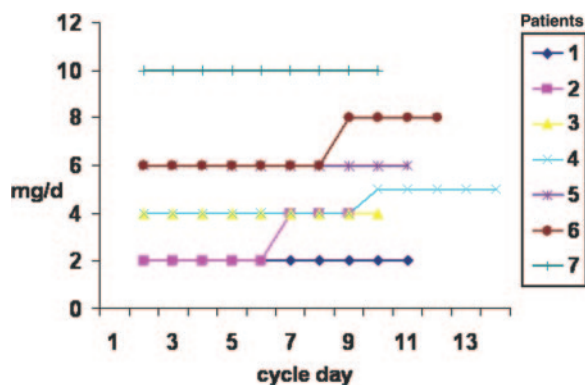


FIG. 1. Daily dose in patients receiving anastrozole. Anastrozole dose plotted against day of controlled ovarian stimulation cycle.  $E_2$  levels at the time of dose increase were 483.4 pg/ml (patient 2), 621 pg/ml (patient 4), and 357.1 pg/ml (patient 6).

**TABLE 1.** Results of COH using letrozole and anastrozole

	Letrozole group (n = 47)	Anastrozole group (n = 7)	P
Age (yr)	36.37 ± 3.4	36.2 ± 4.2	NS
Day 2 FSH (IU/liter)	7.41 ± 3.66	10.27 ± 3.56	NS
Length of stimulation (d)	9.83 ± 2.4	10.7 ± 2.06	NS
Total gonadotropin dose (U)	1469.23 ± 741	1854.36 ± 526.69	NS
E <sub>2</sub> on hCG day (pg/ml)	427.78 ± 278.24 <sup>a</sup>	1325.89 ± 833.17 <sup>b</sup>	<0.01
E <sub>2</sub> on day after hCG (pg/ml)	714.38 ± 440.83 <sup>c</sup>	2515.07 ± 1368.52 <sup>d</sup>	0.01
No. of follicles >17 mm	3.84 ± 1.72	2.67 ± 1.58	NS
No. of oocytes retrieved	11.57 ± 7.14	9.71 ± 8.5	NS
Fertilization rate (%)	74.1 ± 24	71.28 ± 17.11	NS
Embryos cryopreserved	6.19 ± 4.07	5.57 ± 4.86	NS

NS, Not significant.

<sup>a</sup> 1570.38 ± 1021.42 pmol/liter.<sup>b</sup> 4867.34 ± 3058.57 pmol/liter.<sup>c</sup> 2622.49 ± 1618.29 pmol/liter.<sup>d</sup> 9232.82 ± 5023.84 pmol/liter. Conversion factor, 3.471.

### Discussion

In this study we showed that anastrozole had minimal suppressive effect on rising E<sub>2</sub> levels during COH, even at five times the comparable dose of letrozole. As a result, breast cancer patients who underwent ovarian stimulation with anastrozole had a significantly higher exposure to E<sub>2</sub> than those who were stimulated with letrozole.

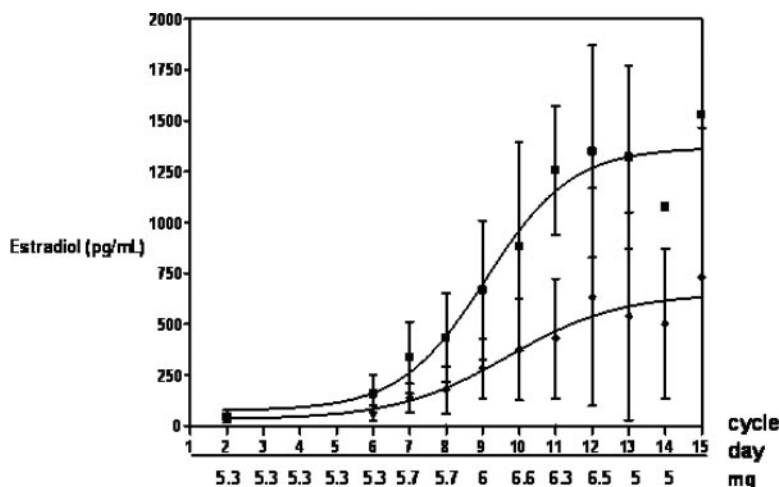
In postmenopausal women, anastrozole has been shown to be subtly inferior to letrozole in inhibiting aromatase activity (8). Probably because estrogen production is already limited, this difference may not become clinically significant. In young women who are undergoing ovarian stimulation on the other hand, there is a rapidly increasing granulosa cell mass in response to ovarian stimulation. Indeed in one study where the pharmacokinetics of anastrozole was tested in reproductive age women, this agent could only suppress E<sub>2</sub> by an average of 39% (16). Furthermore, anastrozole may require 2 wk before significantly inhibiting aromatase activity compared with 2 d with letrozole (17).

Another possible explanation for inadequacy of anastrozole in suppressing E<sub>2</sub> in premenopausal women is the site-specific activity of aromatase inhibitors. Letrozole has been shown to be 80 times more potent in inhibiting aromatase activity in Chinese hamster ovarian cells, compared with

anastrozole (18). Thus, it is possible that the inhibitory effect of letrozole is more specific to granulosa cell aromatase.

Concerns were raised regarding the safety of the use of letrozole for ovarian stimulation. In an abstract presentation, the authors retrospectively compared 150 children born from infertile women to approximately 36,000 children born after spontaneous conception at low risk local labor and delivery room from a non-infertile population (11). The study group was 5 yr older (35 vs. 30) and had much higher multiple birth rates than the general population (15 vs. <1%), both are associated with higher risk of fetal anomalies. Only locomotor abnormalities were found to be higher in the letrozole group. Because infertility patients are inherently different than those who are fertile, an appropriate comparison should have been made to an infertile population. Accordingly, two recent studies compared infertility patients who used letrozole to those who were treated with clomiphene citrate. The first study compared 514 children born after ovarian stimulation with letrozole to 397 born after clomiphene (13). The major malformation rate was not increased in the letrozole-treated group (1.2 vs. 3.0%). Interestingly, there were lower numbers of cardiac abnormalities compared with those who were exposed to clomiphene (0.2 vs. 1.8%; *P* = 0.02). The second study compared 117 children born after their mothers

**FIG. 2.** Mean E<sub>2</sub> levels during stimulation cycles using anastrozole and letrozole. Curve of estradiol-cycle day for anastrozole and letrozole. The curve was constructed by plotting means of E<sub>2</sub> levels in each day of controlled ovarian stimulation cycles followed by curve fitting using nonlinear regression model (GraphPad Prism software). ■, Anastrozole; ♦, letrozole. Mean daily doses of anastrozole are shown below cycle days. Bars represent SD of mean E<sub>2</sub>.



conceived with letrozole treatment to 161 conceived as a result of clomiphene treatment (19). Major anomaly rate with letrozole (2.56%) was similar to that with clomiphene (3.1%).

Thus, although letrozole has been shown to be teratogenic in rodents when exposure occurs during organogenesis (20), there is no clinical evidence that letrozole use is associated with increased birth defects, nor is this biologically plausible in the setting of ovulation induction.

Because of the above data and the findings of this study, we continued to use letrozole to cryopreserve oocytes and embryos from breast cancer patients before chemotherapy. Thus far, there have been six deliveries resulting from the letrozole+FSH protocol used for fertility preservation before cancer treatment. Of 16 embryo transfers to either a surrogate ( $n = 8$ ) or self ( $n = 8$ ), clinical pregnancy and delivery rates were 56.25 and 37.5% per transfer. No patient in the anastrozole group has returned to use her frozen embryos yet. Although these numbers are too small to draw firm conclusions, no birth defects were reported in the six newborns.

In conclusion, anastrozole does not sufficiently suppress  $E_2$  levels in women with breast cancer undergoing controlled ovarian stimulation and thus is not an alternative to letrozole. Because recent studies showed no impact of letrozole on fetal development when used as an ovulation induction agent, research should continue to test the ability of this drug to make embryo or oocyte cryopreservation safer for breast cancer patients.

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Address all correspondence to: Kutluk Oktay, M.D., FACOG, 505 East 70th Street, HT340, New York, New York 10021. E-mail: [koktay@fertilitypreservation.org](mailto:koktay@fertilitypreservation.org). Address requests for reprints to: Kutluk Oktay, Center for Reproductive Medicine, Infertility, Weill Medical College of Cornell University, New York, New York 10021.

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