Letrozole Reduces Estrogen and Gonadotropin Exposure in Women with Breast Cancer Undergoing Ovarian Stimulation before Chemotherapy

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Context: Women with breast cancer are not typically offered embryo or oocyte cryopreservation to preserve their fertility before chemotherapy because of the potential risks associated with high estrogen levels arising from ovarian stimulation.

Objective: We aimed to determine whether the combination of an aromatase inhibitor with gonadotropin treatment in breast cancer patients produces comparable results to standard *in vitro* fertilization (IVF), without a significant increase in estradiol levels and delay in the initiation of chemotherapy.

Patients and Methods: Stages I-IIIA breast cancer patients (n = 47) received 5 mg/d letrozole and 150–300 IU FSH to cryopreserve embryos or oocytes. Age-matched retrospective controls (n = 56) were selected from women who underwent IVF for tubal disease.

Results: Whereas letrozole and FSH stimulation resulted in significantly lower peak estradiol levels (mean $\pm$ SD 483.4 $\pm$ 278.9 *vs.* 1464.6 $\pm$ 644.9 pg/ml; $P < 0.001$) and 44% reduction in gonadotropin requirement, compared with controls, the length of stimulation, number of embryos obtained, and fertilization rates were similar. The human chorionic gonadotropin administration criteria had to be adjusted to 20 mm after letrozole stimulation, compared with 17–18 mm in the controls. The mean delay from surgery to cryopreservation was 38.6 d, with 81% of all patients completing their IVF cycles within 8 wk of surgery.

Conclusion: Ovarian stimulation with letrozole and FSH appears to be a cost-effective alternative for fertility preservation in breast cancer patients with reduced estrogen exposure, compared with standard IVF. If patients are referred promptly, they may undergo embryo or oocyte cryopreservation without a delay in chemotherapy. (*J Clin Endocrinol Metab* 91: 3885–3890, 2006)

I NVASIVE BREAST CANCER is the most common neoplasm encountered during reproductive ages making up one third of all cancers seen in young women (1, 2). More than 15% of all new breast cancer diagnosis occurs under the age of 40 yr (3–6). The majority of these women are given a combination chemotherapy, which includes gonadotoxic agents such as cyclophosphamide. As a result, a significant proportion of cancer survivors suffer from premature ovarian failure and infertility. Most cancer patients would like to preserve their fertility, especially if they have not completed child-bearing (7). Embryo cryopreservation is the most established method of fertility preservation, but it requires approximately 2 wk of ovarian stimulation from the beginning of menses. Whereas that time may not be available for many other invasive cancer types, in breast cancer, there is typically a 6- to 8-wk hiatus between surgery and chemotherapy, allowing sufficient time for undergoing ovarian stimulation for fertility preservation purposes. However, because of the potential risks associated with high estrogen

levels, women with active breast cancer have typically not been offered this option.

Aromatase, an enzyme of the cytochrome P-450 superfamily and the product of the *CYP19* gene, catalyzes the reaction that converts androgenic substances to estrogens in many tissues, including granulosa cells of ovarian follicles (8). Letrozole is a potent and highly selective third-generation aromatase inhibitor that was developed in the early 1990s. It competitively inhibits the activity of aromatase enzyme and has a half-life of approximately 48 h (8). Because of its potent long-lasting suppression in the plasma levels of estradiol (E_2), this drug has recently been claimed to be superior to tamoxifen in the treatment of advanced-stage postmenopausal breast cancer (9–11).

Recent reports have also shown that aromatase inhibitors can be used as ovulation induction agents. In cycling bonnet monkeys, letrozole resulted in the formation of multiple follicles (12). Clinical studies in which letrozole was typically administered at doses of 2.5–5 mg for 5 d have also shown its benefit in ovulation induction alone or in combination with FSH. These studies also showed that peak E_2 levels were lower when letrozole, alone or in combination with FSH, was compared with stimulation with FSH or clomiphene. Moreover, E_2 levels have been found to be even lower than those seen in natural cycle when patients were stimulated with letrozole (13).

We recently developed an ovarian stimulation protocol using letrozole in combination with FSH for the purpose of preserving fertility via embryo or oocyte cryopreservation in breast cancer patients. Our initial short-term follow-up study suggested that a larger number of oocytes than our previous protocol involving tamoxifen can be obtained with this approach without a significant increase in E_2 levels and short-term cancer recurrence rates (8).

In the current study, building on our previous findings, we compared the efficiency of the letrozole+FSH protocol to standard *in vitro* fertilization (IVF) protocols used in patients without breast cancer. Our aim was to determine whether the combination of letrozole with gonadotropin treatment in breast cancer patients produces comparable results with standard IVF, without a significant increase in E_2 levels and delay in the initiation of chemotherapy.

Patients and Methods

This study was approved by the Institutional Review Board of the Weill Medical College of Cornell University (New York, NY). Cancer patients were referred by their medical or surgical oncologists, and patients with stage IV cancer were excluded based on the poor prognosis, general health status of the patient, and the higher uncertainty regarding delaying chemotherapy. Eleven of the 47 patients included in the treatment group were also reported in a previous publication (8). An age-matched control group (n = 56) was retrospectively identified from women who underwent IVF-intracytoplasmic sperm injection (ICSI) with long protocol for tubal factor infertility between the dates of May 2003 and November 2005. Briefly, the stimulation protocol of the control patients included GnRH agonist administration during the luteal phase preceding the ovarian stimulation, with the initiation of gonadotropins on the second or third day of menstrual bleeding using an average dose of 300 U. When two leading follicles reach 17–18 mm diameter, 3,300–10,000 U human chorionic gonadotropin (hCG) is administered, and ultrasound-guided transvaginal retrieval is performed 34–36 h later. The hCG dose was determined based on the peak E_2 levels. Oocytes were fertilized by *in vitro* insemination, except when ICSI was clinically indicated. As a result, ICSI was used in 57.1% of all control cycles.

For patients receiving letrozole+FSH, 5 mg letrozole was administered orally starting on the second or third day of the menstrual cycle as described previously. After 2 d of letrozole administration, 150–300 U/d FSH (Gonal-F or Follistim, Serono Inc., Rockland, MA) was added. hCG was initially administered when leading follicles reached 17–18 mm as per the standard criteria, but because of the high percentage of immature oocytes encountered in initial cycles, this was gradually delayed to 19–21 mm after the treatment of the first two patients with the old criteria (8). Letrozole was discontinued on the day of hCG administration; the E_2 measurement was repeated 3 d after the oocyte retrieval, and if the E_2 level was higher than 250 pg/ml (917 pmol/liter), letrozole was continued until it decreased to less than 50 pg/ml (183 pmol/liter) (8).

Patients were monitored with ultrasound, and the E_2, FSH, and LH measurements were taken every 1–2 d until the day of oocyte retrieval. In the letrozole+FSH group, when E_2 exceeded 250 pg/ml or the leading follicle reached 14-mm diameter, a GnRH antagonist (250 μg, Antagon; Organon Inc., West Orange, NJ) was administered to prevent a premature LH surge. IVF was performed via ICSI, and in all patients the embryos were frozen at the two-pronuclear stage. In one patient, oocytes were cryopreserved without fertilization because she did not have a partner. For each patient, peak E_2 was determined on the day of hCG administration. E_2 measurements were performed by RIA, and FSH measurements were performed by ELISA (Diagnostic Products Corp., Los Angeles, CA).

Statistical analysis

A nonparametric Mann-Whitney-Wilcoxon test was used to compare differences between the two groups (14). Various characteristics were summarized by mean and SD within group. We used data in the first cycle only to avoid dependency among observations. A segmented regression model was used to determine the follicle size break point(s) for hCG administration maintaining the highest maturity and fertilization rate. In many biological models, a relationship between variables may be modeled as a linear or polynomial function that changes abruptly when an independent variable obtains a threshold level or meaningful change points. Usually the transition point is unknown, and a major objective of the analysis is its estimation. A segmented regression model is a class of nonlinear models that intend to characterize this type of complex underlying the relationship between exposure and outcome (15). We determined the optimal number of change points by modified Akaike information criteria (16). Type I error was set at 5% and two-sided hypotheses were adopted for all testing. We considered $P < 0.05$ as statistically significant.

Results

Fifty-seven percent of patients had stage I, 25.5% had stage IIA, 6.4% had stage IIB, and 10.6% had stage IIIA disease. Tumor-node-metastasis (TNM) stage and tumor characteristics of breast cancer patients are shown in Table 1.

The mean delay from the definitive breast surgery to completion of the first IVF cycle was 38.6 ± 14.04 d (mean $\pm$ SD) with a range of 0–79 d. Six patients underwent a second cycle of IVF with the approval of their oncologists, three of them because of the cancellation of the first IVF cycle due to poor response. When second cycles were included, the mean delay was 42.7 ± 21.52 d (mean $\pm$ SD) with a range of 0–102 d. Eighty-one percent of all women completed their IVF cycles within 8 wk of surgery.

Groups were comparable in terms of age (36.4 ± 3.6 *vs.* 36.9 ± 3.9 yr; $P = 0.44$). Mean baseline FSH levels were within normal limits but higher in the letrozole group (7.1 ± 3.1 *vs.* 4.2 ± 2.0, $P < 0.001$). Comparison of cycle characteristics is shown in Table 2. Only the first cycles were included to avoid dependency among observations. Peak E_2 levels were significantly lower with letrozole+FSH, compared with controls. Whereas the length of stimulation was similar, the total amount of gonadotropin used was 44% lower in the letrozole+FSH group, compared with the control group. The mean percentage of mature oocytes was lower with letrozole+FSH, reflecting the high immaturity rate encountered before adjusting the hCG administration criteria. As a result of this adjustment, the mean diameter of all and two

TABLE 1. Tumor characteristics of study participants

	No. of patients[a]	ER positive[b]	PR positive[b]	Her-2/neu positive[b]	With vascular invasion
Stage I	**27 (57.4%)**	**51.2**	**40**	**20**	**12.1**
$T_{1a}N_0M_0$	2	4.7	5	2.5	3
$T_{1b}N_0M_0$	6	9.3	7.5	0	3
$T_{1c}N_0M_0$	19	37.2	27.5	17.5	6.1
Stage IIA	**12 (25.5%)**	**18.7**	**15**	**10**	**9.1**
$T_1N_0M_0$	8	14	12.5	5	6.1
$T_2N_0M_0$	4	4.7	2.5	5	3
Stage IIB	**3 (6.4%)**	**7**	**5**	**5**	**6.1**
$T_2N_1M_0$					
Stage IIIA	**5 (10.6%)**	**9.3**	**10**	**2.5**	**3**
$T_2N_2M_0$					
Total	**n = 47**	**86.04%**	**70%**	**37.5%**	**30.3%**

ER, Estrogen receptor; PR, progesterone receptor.
[a] Percentage of patients in each stage (*bold*) and substage is shown in *parentheses*.
[b] Percentage of all patients showing receptor positivity or vascular invasion in each stage (*bold*) and their distribution in subcategories.

TABLE 2. Comparison of various characteristics between letrozole+FSH and control groups

	Letrozole+FSH[a]	Control[b]	P value
Age at IVF (yr)	36.4 ± 3.6	36.9 ± 3.9	0.44
Baseline FSH	7.1 ± 3.1	4.2 ± 2.0	<0.001
E$_2$ at hCG	483.4 ± 278.9	1464 ± 644.9	<0.001
Endometrial thickness	8.7 ± 2.8	10.9 ± 2.5	<0.001
Follicle no. > 17	4.0 ± 1.7	2.7 ± 1.2	<0.001
Peak follicle size (mm)	21.3 ± 2.6	18.7 ± 1.5	<0.001
Total oocytes (n)	12.4 ± 7.0	11.1 ± 5.5	0.43
Mature oocytes (n)	8.7 ± 4.8	9.7 ± 5.1	0.43
Mature oocytes (%)	73.2 ± 22.9	86.3 ± 12.7	0.003
No. of 2 pn zygotes	6.6 ± 4.0	6.9 ± 4.1	0.73
Fertilization rate	74.1 ± 24.0	73.2 ± 21.5	0.71
No. of days stimulated	11.7 ± 2.3	12.2 ± 1.5	0.09
Total FSH dose	1317.8 ± 578.2	2382.5 ± 1062.8	<0.001

Data represent mean ± SD. pn, Pronuclei.

[a] Forty-seven patients, 47 initiated IVF cycles resulting in 42 retrievals.

[b] Fifty-six patients, 56 initiated IVF cycles resulting in 55 retrievals.

leading follicles at hCG was higher with letrozole+FSH. By the segmented regression analysis, the highest maturity rates were obtained at the 20.4-mm cutoff point for the two leading follicle measurements for letrozole-FSH (Fig. 1A). The regression model with one change point gave the best model fit with Akaike information criteria = 310.13, compared with no or multiple change points models. A strong linear association between follicle size and the maturity rate ($\hat{\beta} = 10 \pm$

3.8, where $\hat{\beta}$ denotes the estimated regression parameter ± SE) is seen before the change point, whereas a slightly decreasing or plateau pattern ($\hat{\beta} = -2.3 \pm 2.7$) was observed after the change point. The slope parameter after the break was not significantly differentiable from zero slope. We repeated the same analysis for the fertilization rate outcome. Positive linearity held up to the change point around 19.5 mm, and the slope became flat after that, where the respective slope estimates correspond to 19 ± 6 and 2.6 ± 2.3 (Fig. 1B). Again, a 95% confidence interval for the slope after the change point includes zero value.

Despite these differences, total number of oocytes and embryos obtained and fertilization rates were similar between the two groups. Endometrial thickness was significantly lower in the letrozole+FSH group, but the mean values remained within a clinically accepted range (17). The latter finding was consistent with the findings from previous studies in which letrozole was used for ovulation induction in noncancer patients (18). A lower percentage of patients underwent ICSI in the control group, compared with letrozole+FSH (57.1 *vs.* 100%, $P < 0.001$), but when comparison was limited to ICSI cycles, the results did not change (data not shown).

Forty-seven cycles were initiated in the letrozole+FSH group. Of the 47, 42 cycles went to oocyte retrieval. Four cycles were canceled due to poor response, and one was discontinued because of a superficial thrombophlebitis in a
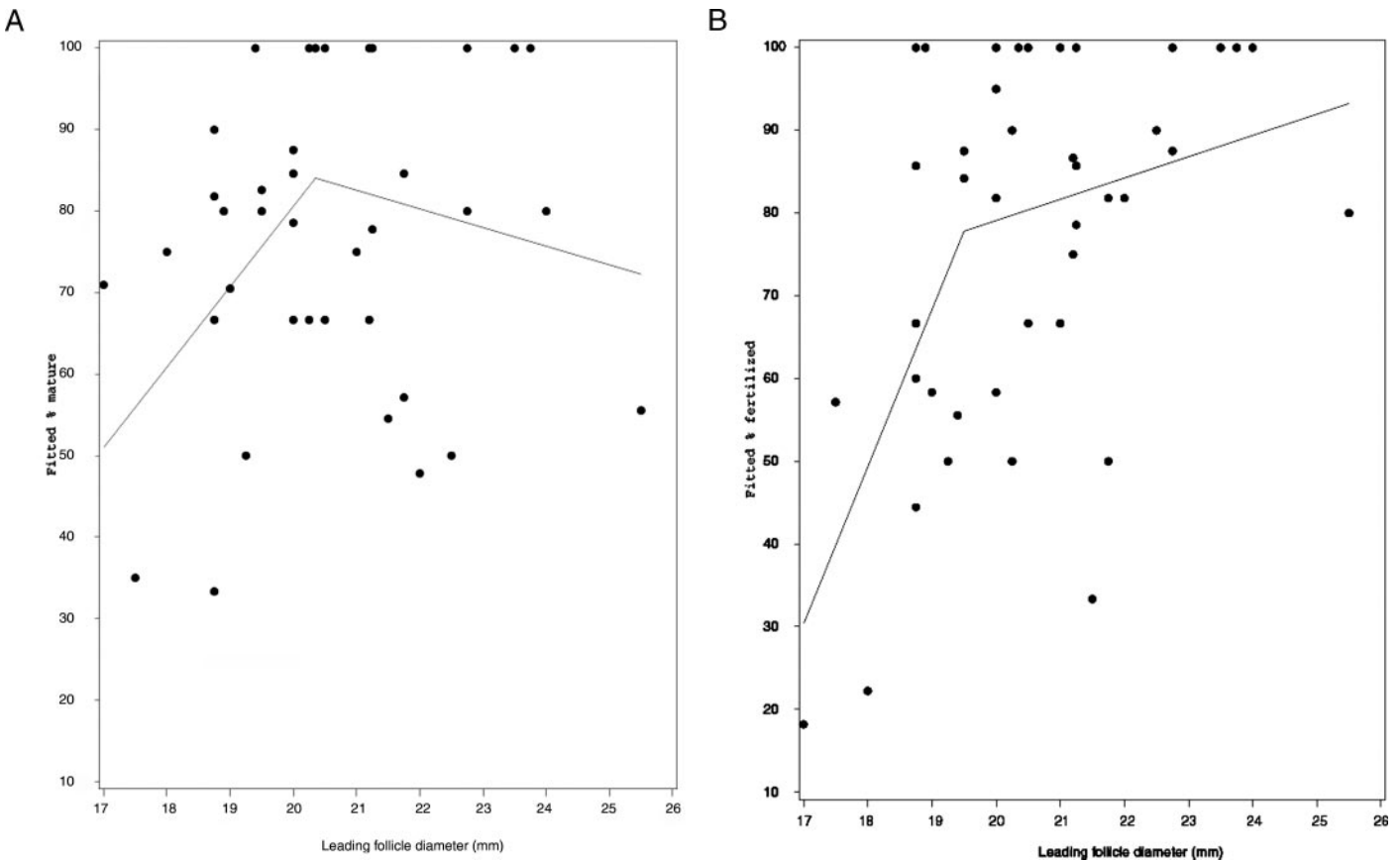

FIG. 1. Segmented regression analysis to determine the optimal hCG administration criteria for maturity rate (A) and fertilization rate (B).

patient's toe. Fifty-six cycles were included in the control group. One was canceled due to poor response.

It was not within the scope of this manuscript to compare the pregnancy rates between the control and treatment groups because all patients in the latter underwent IVF for embryo cryopreservation. At the time of this publication, only three patients had used their frozen embryos resulting from letrozole+FSH stimulation. These resulted in one live birth and one biochemical pregnancy, and one did not conceive. The live birth resulted after thawing three of the seven prezygotic embryos to be transferred to a surrogate. Because all embryos developed to high-grade blastocysts, one was refrozen, and two were transferred. The patient delivered at 36 wk gestation. In addition, two other patients that were not included in this study had fresh embryo transfers after undergoing ovarian stimulation with letrozole+FSH within a year after surgical treatment of early-stage breast cancer. In the first case, three blastocysts were transferred to the patient's uterus, resulting in a singleton live birth at 38 wk gestation. In the other case, the transfer of three cleavage stage embryos resulted in a singleton delivery at 34 wk, but despite the premature delivery, the baby did not experience respiratory distress.

Discussion

Fertility preservation is an important aspect of quality of life in young women with breast cancer. As emphasized in the recent clinical guidelines from the American Society of Clinical Oncology (19), all young patients with cancer should be counseled about the fertility risks of cancer treatments and should be offered appropriate advice on the availability of fertility preservation.

Embryo cryopreservation is an established method that has traditionally been avoided in women with breast cancer due to presumed risks of elevated estrogen levels on cancer cell proliferation. Whereas no study could ethically address whether 2–3 wk of elevated estrogen levels pose a threat to a patient with breast cancer, some evidence exists that this might not be entirely safe. First, acute exposure to estrogen results in increased cell proliferation *in vitro* (20). Second, a large study reported an increase in the incidence of breast cancer in the first year after IVF in otherwise healthy infertility patients (21). However, this risk was normalized during the second to fifth year of follow-up, suggesting either increased surveillance or the possibility of promotion of preclinical cancer by elevated estrogen levels. Supporting the latter hypothesis, we recently observed a high incidence of family history of breast cancer among women who developed breast cancer immediately after IVF treatment (22).

To avoid the possible risks of ovarian stimulation, women with breast cancer have traditionally been offered to undergo natural cycle IVF, which resulted in an extremely low gamete yield. In a recent study, we compared ovarian stimulation with tamoxifen to natural cycle IVF and found that, whereas tamoxifen resulted in a mean 1.6 embryos per patient, natural cycle IVF produced 0.6 embryos per cycle. Whereas all patients had at least one embryo in the tamoxifen group, 40% of cycles did not result in embryo development in the natural cycle IVF group (23).

Our subsequent study compared tamoxifen alone or combined with FSH, with a letrozole+FSH treatment. We found that a larger number of oocytes can be obtained with letrozole+FSH, with a minimal increase in peak E_2 levels. There were no recurrences in the letrozole patients, compared with approximately 10% incidence in tamoxifen-treated patients and the control group, who did not undergo IVF (8). The mean follow-up was 554 ± 31 d with a range of 153-1441 d. Because of these findings, we began to use letrozole+FSH protocol as the primary protocol for women with breast cancer undergoing ovarian stimulation.

In the present study, we showed that when hCG administration criteria is adjusted, similar fertilization rates, number of oocytes, and embryos can be obtained with the letrozole+FSH protocol, compared with standard IVF protocols in women undergoing IVF for mechanical infertility. Moreover, we demonstrated that the best maturity and fertilization results are achieved when hCG is given at 19.5–20.5 mm rather than our traditional criteria of 17–18 mm. A nonlinear modeling technique such as segmented regression successfully estimated the optimal number as well as the locations of the potential change points. However, because the stimulation length was not longer in the letrozole+FSH group, this difference more likely reflects a change in follicular fluid dynamics with letrozole. In fact, an *in vitro* follicle culture study in mice showed that the antral space formed earlier, when follicles are cultured in the presence of an aromatase inhibitor, compared with controls, but the oocyte competency was not altered (24).

To ensure that the control group of patients is similar to the otherwise healthy breast cancer patients undergoing fertility preservation, we only included those with tubal factor infertility. This enabled us to avoid confounding from factors such as low ovarian reserve or severe male factor in the control group. This approach resulted in a lower percentage of patients undergoing ICSI in the control group as ICSI was performed in all cancer patients undergoing fertility preservation. Nevertheless, fertilization and pregnancy rates do not differ between patients undergoing IVF with insemination *vs.* ICSI, except for most severe male factor infertility patients in our program, and the comparison of letrozole+FSH patients with those who underwent ICSI in the control group did not change our results.

Another finding from this study was that whereas similar numbers of embryos were obtained, the addition of letrozole reduced the need for gonadotropins by 44%. Considering the high cost of injectable gonadotropins, this could mean a significant savings for cancer patients who are already stretched with the expense associated with the treatment of their primary disease.

Our findings are consistent with the work of other investigators. In one study in which a similar but not identical protocol involving letrozole, FSH, and GnRH antagonist was used in poor responders undergoing IVF treatment (25), relatively low levels of estrogen on the day of hCG, equivalent fertilization rate, compared with FSH alone, but a higher implantation rate in the letrozole/FSH group was encountered. In another study, when a 5-d protocol of letrozole was combined with gonadotropins for controlled ovarian hyperstimulation in infertility patients, gonadotropin re-

quirement was also reduced compared with standard protocols (26).

We also found that most women with breast cancer can undergo ovarian stimulation without delaying their chemotherapy because there is a 6- to 8-wk hiatus between the surgery and initiation of chemotherapy. In our study, average time from surgery to initiation and completion of IVF was 27 and 42 d, respectively, with more than 80% of patients completing their treatment within 8 wk of surgery. Because we also found that these patients are delayed on average for 42 d from the diagnosis to referral for fertility preservation, a more expedited referral approach is needed by the oncologists to avoid postponement of chemotherapy.

A recent abstract questioned the safety of the use of aromatase inhibitors for ovulation induction (27). In that abstract, the authors retrospectively compared 150 children born from infertile women with approximately 36,000 children born after spontaneous conception from a noninfertile population of women at a low-risk local labor and delivery room. The study group was 5 yr older (35 *vs.* 30), and they had much higher multiple birth rates than the general population (15 *vs.* <1%). Both the older age and multiple gestation are associated with higher risk of fetal anomalies. Moreover, in the letrozole group, 21 children were lost to follow-up, raising the possibility of negative recall bias. Only the locomotor abnormalities were found to be higher in the letrozole group. Their results are yet to be published in a peer-reviewed journal.

Because infertility patients are inherently different from those who are fertile, an appropriate comparison should have been made with an infertile population. Accordingly, two recent studies compared infertility patients who used letrozole with those who were treated with clomiphene citrate. The first study compared 514 children born after ovarian stimulation with letrozole with 397 born after clomiphene (28). The major malformation rate was not increased in the letrozole-treated group (1.2 *vs.* 3.0%). Interestingly, there were lower number 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 conceived with letrozole treatment with 161 conceived as a result of clomiphene treatment (29). Major anomaly rate with letrozole (2.56%) was similar to that with clomiphene (3.10%).

Exposure of oocytes to aromatase inhibitors is also not associated with aberrations in oocytes or the offspring. In a recent study, aromatase-overexpressing mice were treated with high doses of letrozole for 6 wk, and the animals were allowed to mate 2 wk after the last dose. There was no difference in the litter size, birth weight, or number of anomalies in pups born to letrozole-exposed mice, compared with control animals (30). Moreover, when oocytes were exposed to another aromatase inhibitor, anastrozole, there was no increase in spindle anomalies, and the rate of development to blastocysts was similar to controls (24). Furthermore, when used for ovulation induction, letrozole is expected to be cleared from the circulation by the time embryos implant because it has a 48-h half-life (31); and when embryos are cryopreserved, they cannot practically be exposed to letrozole because the fertilization takes place *in vitro*. Thus,

whereas letrozole has been shown to be teratogenic in rodents when fetuses are exposed during organagenesis (32), there is neither clinical evidence that letrozole use is associated with increased birth defects nor is this biologically plausible in the setting of ovulation induction.

The majority of the patients who underwent ovarian stimulation with letrozole+FSH have not yet returned for embryo transfer, and thus, we are unable to compare the pregnancy success rates with standard IVF protocols. However, we have had a small number of patients who attempted pregnancy with either frozen or fresh embryos after breast cancer treatment. Four of five patients conceived, and of those who conceived, three delivered and one had an early miscarriage. Nevertheless, to further certify that the letrozole+FSH protocol is at least similarly successful as standard IVF protocols, embryo transfer data with larger number of patients will be needed.

In summary, letrozole+FSH protocol appears to result in similar oocyte and embryo yield as standard IVF protocols with reduced estrogen exposure and cost. The majority of patients can complete their cycles without a significant delay in chemotherapy. However, patients should be referred for fertility preservation more expeditiously to avoid potential delays in treatment. Whereas our earlier work suggested the short-term safety of ovarian stimulation with letrozole+FSH, the long-term safety of this approach in breast cancer patients will have to be determined in follow-up studies with larger number of patients. More speculatively, further research will determine whether letrozole can be used to lower the cost and estrogen exposure in infertility patients undergoing IVF without the history of breast cancer.

Acknowledgments

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