

Safety of Fertility Preservation by Ovarian Stimulation With Letrozole and Gonadotropins in Patients With Breast Cancer: A Prospective Controlled Study

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A B S T R A C T

Purpose

Because of the accompanying increase in estrogen levels, safety of performing in vitro fertilization (IVF) in women with breast cancer is unknown. Our goal was to determine the effect of controlled ovarian stimulation (COS) using a combination of letrozole with standard fertility medications on disease-free survival in women undergoing embryo or oocyte cryopreservation before adjuvant chemotherapy.

Patients and Methods

A total of 215 women with breast cancer were prospectively evaluated for fertility preservation before adjuvant chemotherapy. Of those, 79 elected to undergo COS with letrozole and gonadotropins for embryo or oocyte cryopreservation. The remaining 136 patients underwent no fertility-preserving procedure and served as controls.

Results

Study and control groups were similar at enrollment except for a trend for higher estrogen-receptor positivity in the COS group ($P = .08$). Time between surgery and chemotherapy was longer for IVF patients (45.08 v 33.46 days; $P < .01$). Peak estradiol levels ranged from 58.4 to 1,166 pg/mL (mean, 405.94 ± 256.64 pg/mL or $1,486.76 \pm 942.13$ pmol/L) in COS patients. The median follow-up after chemotherapy was 23.4 months (range, 7.5 to 63.6 months) in the COS group and 33.05 months (range, 4.5 to 63.6) in the control group. The hazard ratio for recurrence after IVF was 0.56 (95% CI, 0.17 to 1.9), and the survival was not compromised compared with controls ($P = .36$).

Conclusion

Ovarian stimulation with gonadotropins and letrozole for the purpose of fertility preservation is unlikely to cause substantially increased recurrence risk. Further research, including longer-term follow-up is needed to confirm these findings.

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INTRODUCTION

It is estimated that invasive breast cancer will have affected approximately 178,000 women in the United States in the year 2007,¹ of whom approximately 22% are expected to be younger than 50 years of age.² Worldwide, 240,000 women are diagnosed with breast cancer before the age of 44 years, according to GLOBOCAN database.³ The majority of these women will receive adjuvant or neoadjuvant chemotherapy,⁴ which has a potential significant impact on future fertility.

The use of adjuvant/neoadjuvant chemotherapy is commonly associated with amenorrhea and marked reduction of ovarian reserve depending on the patient's age, class of the agents, and dose.⁵⁻¹⁰ This diminution in ovarian reserve is compounded

by the need to delay pregnancy while undergoing hormonal treatment (tamoxifen with or without gonadotropin-releasing hormone [GnRH] agonists) for 5 years or longer in estrogen receptor-positive diseases.

The 5-year survival in all stages of breast cancer has reached 89% in the United States.¹¹ As a result of the high survival rates and the increased emphasis on the quality of the life of the survivor, fertility preservation is gaining importance. As stressed in the recent clinical guidelines by the American Society of Clinical Oncology, all cancer patients with interest in future fertility should be referred for consideration of fertility preservation.¹²

Both embryo and oocyte cryopreservation require controlled ovarian stimulation (COS). Whereas embryo cryopreservation is an established

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and widely available method since 1983, oocyte cryopreservation is still investigational, with fewer than 1,000 babies born worldwide, but the success rates are improving.¹³ With embryo cryopreservation, the average live birth rate is 27.7% per embryo transfer cycle in the United States.¹⁴ Ovarian stimulation entails the use of gonadotropin injections to cause multiple follicular recruitment followed by transvaginal oocyte retrieval. In addition, GnRH agonists or antagonists are administered to prevent premature luteinization and ovulation during COS. The use of COS is associated with marked increase in estradiol (E_2) levels,¹⁵ sometimes 10 to 20 times of the levels seen in natural cycles.¹⁶ Because of the large body of data implicating estrogen and estrogen metabolites in breast cancer propagation,¹⁷ patients and oncologists are reluctant to accept the use of traditional ovarian stimulation regimens because of their association with a rise in E_2 levels.¹⁸⁻¹⁹

To avoid the potential risks of rising of E_2 levels during COS, we previously developed an ovarian stimulation protocol utilizing the aromatase inhibitor letrozole before in vitro fertilization (IVF) for embryo or oocyte cryopreservation.²⁰⁻²³ We found that E_2 levels may remain at levels similar to unstimulated cycles while aromatase inhibitors are used concurrent with gonadotropin injections, yet the oocyte and embryo yield are comparable to those of standard ovarian stimulation protocols. Nevertheless, the general acceptance of this approach requires that its impact on the course and natural history of breast cancer be fully understood.

The aim of the current study was to determine whether ovarian stimulation with concurrent use of gonadotropins and letrozole before chemotherapy affects breast cancer recurrence rates, and hence the prognosis.

PATIENTS AND METHODS

Study Patients

This prospective nonrandomized controlled study was conducted between January 2002 and April 2007. The study was approved by the institutional review board and was registered at clinicaltrials.gov (NCT00504699). The study population consisted of those breast cancer patients who were referred for consultation for fertility preservation before adjuvant chemotherapy. All patients fitting the inclusion criteria were offered COS with letrozole and gonadotropins, IVF, and embryo or oocyte cryopreservation. Those who did not wish to undergo fertility preservation but who agreed to a follow-up by phone served as controls. Some of the patients in the study ($n = 43$) and control groups ($n = 44$) were included in a prior publication with much shorter duration of follow-up.²¹ The treatment group underwent fertility preservation by embryo or oocyte cryopreservation (depending on whether a partner existed) after COS with a combined letrozole-gonadotropin protocol followed by oocyte retrieval. The inclusion criteria were age 18 to 45 years, histologically confirmed invasive breast carcinoma, and normal basal (menstrual cycle day 2 or 3) follicle-stimulating hormone (FSH) (<13 mU/mL) and E_2 less than 75 pg/mL (equivalent to 275.3 pmol/L). Only women with stage III cancer or less were enrolled in the study. Four patients were not eligible because of advanced stage disease ($n = 2$) or age older than 45 years ($n = 2$). Pathology reports were reviewed to confirm the type of breast surgery, tumor histology, size, grade, lymph node status, estrogen and progesterone receptor, and human epidermal growth factor receptor (HER)-2/*neu* (overexpression of epidermal growth factor receptors) status. The interval between the definitive surgery and the onset of chemotherapy was recorded for each patient.

Follow-Up

Follow-up information was collected either during return visits, by phone interview, or by contacting the patient's referring oncologist. Recurrence was defined as the detection of locoregional tumor (chest wall, regional

nodal disease), distant metastases, or contralateral invasive breast cancer. Time to recurrence was calculated from the time of definitive surgery. Patients were censored when lost to follow-up or at the last time point their disease status was known.

Ovarian Stimulation

Ovarian stimulation was performed utilizing letrozole (Femara, Novartis, East Hanover, NJ), in combination with gonadotropins as previously described.²⁰⁻²² Letrozole was started at 5 mg/d dose on the second day of menstrual cycle and continued until the day of human chorionic gonadotropin (hCG) trigger.²⁰⁻²¹ Daily injections of recombinant FSH (Follistim, Organon, West Orange, NJ or Gonal-F, Serono, Rockville, MD) with or without human menopausal gonadotropin (hMG; Repronex, Ferring, Tarrytown, NY) were added 2 days after the initiation of letrozole. The starting dose of FSH ranged between 150 and 300 U, and between 0 and 150 U for hMG. To prevent premature luteinizing hormone (LH) surge, a GnRH antagonist (Ganirelix 250 μ g/d, Organon, West Orange, NJ) was administered when serum E_2 levels were at least 250 pg/mL (equivalent to 918 pmol/L) or when the lead follicle size reached 14 mm in mean diameter. The gonadotropin dose was determined on the basis of the patient's age and body mass index according to standard protocols in our department. After a baseline pelvic ultrasound assessment on cycle day 2, ultrasound and E_2 monitoring were performed every 1 to 2 days after the initiation of gonadotropins. hCG was administered intramuscularly when at least two follicles reached at least 19 to 20 mm in diameter. Transvaginal oocyte retrieval was performed approximately 36 hours after hCG administration. Oocytes were fertilized by intracytoplasmic sperm injection. Embryos were cryopreserved by slow freezing at the prezygote (two-pronuclei) stage. If oocyte cryopreservation was to be performed, oocytes and surrounding cumulus were frozen by slow freezing technique with propanediol as cryoprotectant. Letrozole was reinitiated on the day of oocyte retrieval to prevent a rebound increase in E_2 levels and continued until E_2 was less than 50 pg/mL (equivalent to 183.5 pmol/L; Figure 1).

Hormone Analysis

FSH and LH were measured using a solid-phase chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA). The FSH assay has a sensitivity of 0.1 mU/mL and the LH assay has a sensitivity of 0.05 mU/mL. Serum E_2 was measured by an in-house radioimmunoassay (Direct 125I; Pantex, Santa Monica, CA). The assay has a minimum sensitivity of 10 pg/mL, intra-assay coefficient of variation of 4.2% to 16% and interassay coefficient of variation of 7.3% to 15.5%.

Statistical Analysis

Patients' demographic characteristics, hormonal data, and IVF outcomes were presented as mean $\pm$ SD. *t* test was used for comparison of population means and χ^2 for comparison of proportions. $P < .05$ (two sided) was considered statistically significant. Whenever possible, 95% CIs were calculated. Ten-year breast cancer mortality and relapse were estimated for each patient taking in consideration age, size of tumor, grade, number of

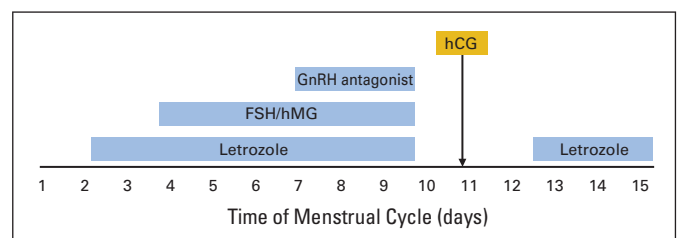


Fig 1. Protocol for ovarian stimulation with letrozole and gonadotropins in patients diagnosed with breast carcinoma. In this regimen, letrozole is initiated on the second day of menstrual cycle and gonadotropins are started 2 days later. A gonadotropin-releasing hormone (GnRH) antagonist is administered when estradiol levels reach ≥ 250 pg/mL or the lead follicle size reaches 14 mm. Human chorionic gonadotropin (hCG) is administered when the leading follicle reaches 19 to 20 mm in diameter. Letrozole treatment is restarted after oocyte retrieval until the estradiol levels are lower than 50 pg/mL. FSH, follicle-stimulating hormone; hMG, human menopausal gonadotropin.

lymph nodes, estrogen-receptor status, use of tamoxifen and chemotherapy regimen used. Mortality and relapse risks were calculated using Adjuvant! Online, standard version 8.0 (www.adjuvantonline.com).²⁴ Survival analyses for recurrence were performed using the Kaplan-Meier method and compared using the Mantel-Haenszel (log-rank) test. If patients were not available for follow-up, they were considered free of disease in the control group and counted as recurrence in the study group. Cox proportional hazard regression model was used to compare the effects of multiple prognostic covariates between both groups on survival analysis. A priori power analysis indicated that 80 patients were required in each arm to detect 10% difference in disease-free survival between the groups with a power of 80%. Prism4 software (Graphpad Inc, San Diego, CA) was used for statistical analysis. SPSS (version 12, Chicago, IL) was used for conducting Cox regression analysis.

RESULTS

A total of 215 women with breast cancer were evaluated for fertility preservation before adjuvant chemotherapy. Of those, 79 elected to undergo COS with letrozole and gonadotropins for embryo or oocyte cryopreservation. The remaining 136 patients who elected to not to undergo ovarian stimulation because of general concern about safety, cost, or delay of chemotherapy, were later contacted to serve as controls. Patient characteristics are presented in Table 1. All 79 patients in the study group and 102 (75%) of 136 patients in the control group were reached and orally consented for follow-up. This difference in follow-up would have biased only toward the detriment of the treatment group because those who were lost to follow-up were considered disease-free in the control group.

The two groups were similar in terms of age at diagnosis, breast cancer prognostic parameters (tumor size, grade, and number of positive lymph nodes, HER-2/*neu* overexpression, and vascular space invasion), use of chemotherapy, and postchemotherapy use of GnRH agonist. There was, however, a trend for higher estrogen-receptor positivity in the COS group ($P = .08$). One patient in the study group underwent risk-reducing bilateral salpingo-oophorectomy for a positive *BRCA2* breast cancer gene mutation. There was no difference between the two groups in the projected 10-year relapse, breast cancer-specific mortality, or overall mortality (using Adjuvant! online) at enrollment onto this study.

Time between surgery and chemotherapy was longer for patients undergoing IVF (45.08 ± 31.64 v 33.46 ± 27.3 days; $P < .01$). Length of stimulation was 9.87 ± 2.28 days (range, 7 to 16 days). Peak E_2 levels ranged from 58.4 to 1,166 pg/mL with a mean of 405.94 ± 256.64 pg/mL (equivalent to 1486.76 ± 942.13 pmol/L) in COS patients. An average of 10.3 ± 7.75 oocytes was retrieved, and 5.97 ± 4.97 embryos or oocytes cryopreserved per patient. The median length of follow-up after definitive surgery was 23.4 months (range, 7.5 to 63.6 months) in the study group and 33.05 months (range, 4.5 to 63.6 months) in the control group ($P < .0001$). There were three (3.8%) recurrences or contralateral breast cancers (two distant, one locoregional) in the letrozole group, and 11 (8.1%) in the control group (nine distant, one locoregional, one contralateral breast; $P = .26$). There was no significant difference in relapse-free survival between the groups (Kaplan-Meier method $P = .36$; hazard ratio = 0.56; 95% CI, 0.17 to 1.9; Fig 2).

Cox regression analysis was used to study the difference in the effect of tumor size, grade, number of lymph nodes, vascular space invasion, estrogen- and progesterone-receptor status, overexpression of HER-2/*neu*, surgery performed (breast-conservation surgery and total mastectomy), type of chemotherapy used (anthracycline based,

cyclophosphamide + methotrexate + fluorouracil and similar regimens, others), the use of tamoxifen and length of follow-up (together or successively) on relapse-free survival between both groups. The effect of each of these variables was not significantly different in the study group compared with controls.

Only a small fraction of the patients attempted pregnancy during the follow-up period of this study. Ten patients in the COS group underwent embryo replacement to their own uterus ($n = 4$) or to a gestational carrier ($n = 6$) after breast cancer treatment, resulting in eight pregnancies and five deliveries. One spontaneous pregnancy occurred in that group, and is currently ongoing. In the control group, three spontaneous pregnancies occurred. Although one resulted in a live birth, the other two miscarried. We did not observe any deleterious effect to the use of letrozole on embryo quality before or after thawing.

DISCUSSION

Fertility preservation is a priority for young women with breast cancer. However, the concern of estrogen exposure during fertility preservation treatments limit the access of many survivors to established procedures such as embryo cryopreservation. In this study, we demonstrated that when ovarian stimulation was performed with concurrent use of an aromatase inhibitor, the relapse-free survival rate was unlikely to be affected, after a median follow-up of approximately 2 years. On the basis of these data, ovarian stimulation with letrozole and FSH seems to be safe in women with breast cancer, at least in the short term.

One of the concerns with ovarian stimulation before breast cancer chemotherapy is the potential delay in the initiation of treatment. Ovarian stimulation takes approximately 2 weeks from the beginning of menstruation. As expected, in our study, the lag period between definitive surgery and start of chemotherapy was significantly longer in the IVF group compared with the control group. Multiple studies have demonstrated no effect on survival or recurrence if chemotherapy was started 12 weeks or less after surgery.^{25,26} This finding thus is probably not considered clinically meaningful because the lag time was within the clinically acceptable range and considered standard. Nevertheless, there is a need to increase the awareness with fertility preservation techniques so that the patients are referred as early after the initial diagnosis as possible.

Follow-up information was available for all cases in the ovarian stimulation group versus 75% of controls. Patients in the control arm were less likely to speak to investigators about their disease status. One could speculate that this was probably the result of a lower interest in fertility preservation issues than the stimulation group. Nonresponders in the control group were considered disease free for calculations of recurrence and survival; thus, this difference in response rates would have only overestimated the recurrence risk in the IVF group. A significantly higher proportion of patients were receiving tamoxifen in the study group. However, we adjusted for this difference in Cox model. Use of trastuzumab for HER-2/*neu*-positive tumors was not included in the regression model because of lack of published information on long-term (> 2 years) effects on survival and relapse.²⁷ HER-2/*neu* status and use of trastuzumab is not included in Adjuvant! online for the same reason. Thus we were

Table 1. Patient Characteristics, Breast Cancer Prognosis, and Treatment

Characteristic	Treatment Group (n = 79)		Control Group (n = 136)		P	95% CI
	No.	%	No.	%		
Age at diagnosis, years						
Mean		36.1		35.6	.4	
Standard deviation		3.8		4.7		
Median		36		35		
Surgery						
Less than total mastectomy	50	63.3	85	62.5	.99	0.71 to 1.47
Total mastectomy	29	36.7	51	37.5		
Nodal status						
Negative	49	62	86	65.2	.93	0.82 to 1.25*
1-3 positive nodes	26	32.9	42	30.9	.99	0.86 to 1.24
≥ 4 positive nodes	4	5.1	8	5.9		
Tumor size, cm						
Mean		1.79		1.66	.42	
Standard deviation		1.04		1.15		
Median		1.5		1.5		
0.1-2	58	73.4	110	80.8	.23	0.78 to 1.06
2.1-5	20	25.3	24	17.7		
>5	1	1.3	2	1.5		
Histologic grade						
1-2	49	62	89	65.4	.47	0.80 to 1.63
3	30	38	47	34.6		
Lymphovascular space invasion	19	24.1	25	18.4	.38	0.77 to 2.22
Estrogen-receptor positive	64	81	95	69.9	.08	0.99 to 1.35
HER-2/ <i>neu</i> positive	21	26.6	35	25.7	.99	0.65 to 1.64
Adjuvant tamoxifen use	52	65.8	63	46.3	.047	1.02 to 1.48
Adjuvant trastuzumab use	11	13.9	14	10.3	.42	0.74 to 2.33
GnRH agonist	18	22.8	19	14	.13	0.96 to 2.09
Chemotherapy						0.57 to 1.92†
None	14		23		.99	
AC	8		29			
ACT	47		57			
CMF	5		10			
CEF	2		2			
Others	3		15			
History of ovulation induction	13		7			
Clomiphene	5		4			
Gonadotropins	8		3			
10-year relapse rate‡					.44	
Estimate		20.38		21.52		
Standard deviation		10.59		9.23		
10-year specific mortality‡					.85	
Estimate		11.67		11.92		
Standard deviation		9.59		8.22		
10-year overall mortality‡					.88	
Estimate		12.86		13.6		
Standard deviation		9.39		7.94		

Abbreviations: GnRH, use of gonadotropin releasing hormone agonist after chemotherapy; AC, doxorubicin/cyclophosphamide; ACT, doxorubicin/cyclophosphamide plus a taxane; CMF, cyclophosphamide/methotrexate/fluorouracil; CEF, cyclophosphamide/epirubicin/fluorouracil.

*Comparison between positive and negative nodes.

†Comparison between adjuvant chemotherapy and none.

‡Average percentages of relapse, mortality and overall mortality.

not able to calculate the effect of trastuzumab use on overall and disease-free survival.

We could not accurately estimate the rate of long-term amenorrhea after chemotherapy because some patients in both groups were still receiving hormonal treatment (eg, GnRH agonists) or less than a year had elapsed since the last chemotherapy cycle.

Although there has been no clear correlation between incidence of breast cancer and fertility treatments,²⁸ some studies showed a transient increase in risk of breast cancer after IVF treatments.²⁹ Others showed a borderline increase in risk in patients with positive family history,^{30,31} or in those who used gonadotropins for at least 6 months.³² An association between breast cancer with poor prognostic

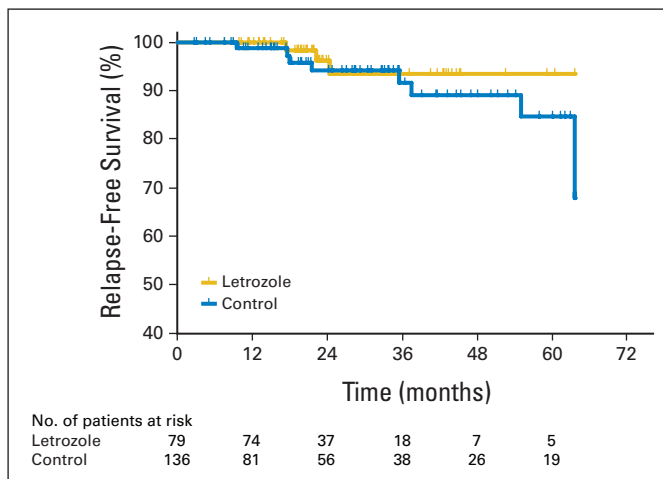


Fig 2. Relapse-free survival in ovarian stimulation and control groups. Kaplan-Meier plot for relapse-free survival in letrozole and control groups. $P = .36$ (log-rank test), hazard ratio = 0.56. The number of patients at risk at each year is shown below the graph.

features and ovarian stimulation has also been suggested.³³ Furthermore, the result of a recently published and the largest study to date indicates that gonadotropins may have a stronger effect on breast cancer risk among nulliparous women.³⁴ These data convey sufficient concern regarding the use of standard ovarian stimulation protocols in young women diagnosed with breast cancer. If standard IVF treatments increase breast cancer risk in general infertility population, it would only be logical to assume that they may have an impact on the course of cancer in women with active breast malignancies. Our study, however, was not designed to address whether the standard IVF treatments increase the risk of breast cancer among infertile patients without cancer.

Even though aromatase inhibitors are contraindicated during pregnancy,³⁵ there is no credible evidence that their use before conception poses any risk to the fetus.^{36,37} Moreover, when used for fertility preservation by cryopreservation, embryos are never exposed to the drug because the fertilization takes place in vitro and they are not transferred to the uterus in the cycle that letrozole is used.

Our study has several limitations. This is not a randomized study; however, a randomized trial of this type would not be possible, given the recognized impact of chemotherapy on fertility as well as the unknown effects of standard ovarian stimulation protocols on breast

cancer prognosis. Despite lack of random assignment, the study and control groups were similar and they did not differ in age, breast cancer prognostic parameters and projected 10-year relapse, and breast cancer-specific and overall mortality rates. The relatively small sample size and pattern of referral from oncologists probably contributed to uniformity between stimulation and control groups. The study does not provide long-term follow-up; the median follow-up period after surgery is approximately 2 years (range, 0.63 to 5.3 years). Even though the highest risk for recurrence occurs within the first 2 years after the treatment,³⁸ patients with estrogen receptor-positive cancers tend to experience recurrence more than 5 years after treatment. This should be considered in interpreting the power of the study. We will continue our study to obtain follow-up information for up to 10 years to exclude the potential long-term effects of ovarian stimulation.

In conclusion, our study suggests that the use of letrozole and gonadotropins for controlled ovarian stimulation before embryo or oocyte cryopreservation is unlikely to result in a significant increase in recurrence of breast cancer compared with those who did not undergo ovarian stimulation, at least in the short term. Because of the relatively short-term follow-up and small number of patients, however, a definitive judgment on the safety of our approach cannot yet be rendered. Further follow-up will be needed to determine whether this ovarian stimulation protocol has an impact on long-term recurrence or survival. In the meantime, utilization of letrozole-FSH protocol in women with breast cancer who wish to preserve their fertility by oocyte or embryo cryopreservation is certainly a viable option.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Kutluk Oktay

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Manuscript writing: Amr A. Azim, Kutluk Oktay

Final approval of manuscript: Kutluk Oktay

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