Safety and feasibility of performing two consecutive ovarian stimulation cycles with the use of letrozole-gonadotropin protocol for fertility preservation in breast cancer patients

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Objective: To investigate the safety and feasibility of performing two consecutive ovarian stimulation cycles with the use of letrozole protocol for fertility preservation in breast cancer patients.
Design: Retrospective cohort study.
Setting: Academic fertility preservation center.
Patient(s): Seventy-eight women ≤45 years, diagnosed with stage ≤3 breast cancer, who desired fertility preservation.
Intervention(s): Two consecutive cycles versus a single ovarian stimulation cycle with a letrozole-follicle–stimulating hormone (FSH) protocol.
Main Outcome Measure(s): Embryo or oocyte cryopreservation outcomes, time interval from surgery to chemotherapy, and breast cancer recurrence rates.
Result(s): Sixty-one patients underwent single-cycle stimulation and 17 received two stimulation cycles. The mean total number of oocytes harvested (16.1 ± 13.2 vs. 9.1 ± 5.2) and embryos generated (6.4 ± 2.9 vs. 3.7 ± 3.1) were statistically significantly higher in patients who underwent two cycles versus one cycle. The time interval from surgery to chemotherapy was similar between the two-cycle and single-cycle groups (63.7 ± 7.7 vs. 58.0 ± 12.1 days). After a mean follow-up interval of 58.5 ± 13.6 months, the recurrence rates were similar between the two-cycle (0 of 17) and single-cycle (2 of 49) patients.
Conclusion(s): It appears to be safe and feasible to perform two consecutive ovarian stimulation cycles to increase the oocyte/embryo yield for fertility preservation. (Fertil Steril® 2013;100:1681–5. ©2013 by American Society for Reproductive Medicine.)
Key Words: Breast cancer, consecutive cycles, fertility preservation, letrozole, ovarian stimulation

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Breast cancer is the most prevalent malignancy among reproductive-aged women in the United States (1). With improvements in diagnostic and therapeutic strategies, breast cancer mortality rates have significantly declined over the past several years (2). At present, breast cancer survivors represent the largest

group of cancer survivors in the United States (3). The new challenges that these survivors now face are chemotherapy-induced ovarian reserve damage, premature ovarian failure, and infertility.

Reproductive endocrinologists should counsel and provide information about fertility preservation options to breast cancer patients who will be facing such gonadotoxic treatments. Embryo, oocyte, and ovarian tissue cryopreservation are the three main options to preserve fertility, although ovarian tissue cryopreservation is still experimental (4). Both embryo and oocyte cryopreservation require controlled ovarian hyperstimulation (COH). However, traditional COH regimens are associated with higher levels of estrogen and as a result are not recommended for breast cancer patients. To protect the patients from the potential deleterious effects of elevated estrogen levels during ovarian stimulation for fertility preservation, protocols using aromatase inhibitors were developed (5). Letrozole, a potent and highly selective third-generation aromatase inhibitor, has been shown to reduce estrogen exposure when combined with gonadotropin for ovarian stimulation in breast cancer patients (6). Letrozole reduces serum estrogen levels by aromatase inhibition; because of the reflex increase in endogenous follicle-stimulating hormone (FSH), aromatase inhibitors also result in ovarian stimulation.

Women with breast cancer typically have an interval of 6 to 8 weeks between surgery and the initiation of adjuvant chemotherapy. However, studies have shown no effect on survival or recurrence rates in patients with early stage breast cancer if chemotherapy is initiated 12 weeks after breast surgery (7, 8). Early fertility preservation referral enables earlier counseling and initiation of cryopreservation cycles before chemotherapy; when appropriate, two consecutive ovarian stimulation cycles may be completed without any delay in chemotherapy treatment. For instance, patients who have had oocyte retrieval within 4 weeks of the breast surgery are able to complete a second ovarian stimulation cycle within 8 weeks of surgery. Because of this early counseling and initiation of fertility preservation, these women are at an advantage because a greater number of oocytes and embryos can be cryopreserved. Thus, the chemotherapy-induced ovarian reserve loss may be better compensated by cryopreserving a higher number of oocytes and embryos. Supporting this notion, we showed in a recent individual patient data meta-analysis that the live-birth probability from in vitro fertilization (IVF) increases with the increasing number of oocytes frozen from individuals, regardless of age (9).

In this study, we investigated the safety and feasibility of performing two consecutive ovarian stimulation cycles for fertility preservation before the initiation of chemotherapy among breast cancer patients. We also analyzed the interval differences in time of surgery and the initiation of chemotherapy between two groups: those receiving two versus one ovarian stimulation cycle.

MATERIALS AND METHODS

Institutional review board approval was obtained at New York Medical College. Data were generated via a secondary analysis of a prospective database of all women diagnosed with breast cancer who underwent assisted reproductive technology (ART) treatment at our institution. The exclusion criteria included age >45 years, breast cancer stage >3, previous chemotherapy or radiotherapy, and history of ovarian surgery or infertility (Supplemental Fig. 1, available online).

Recruitment within the single cycle and two consecutive cycles therapy groups was not randomized. The decision to undergo a single cycle or two consecutive cycles was based on several factors, including the amount of time available from referral to chemotherapy start date, the patient's desire to increase the total number of oocytes/embryos, and the concordance of the patient's oncologist.

The letrozole protocol used for ovarian stimulation was identical for both groups. Letrozole at 5 mg/day was started on cycle day 2 or 3. Daily injections of FSH (150–300 IU/day) were added beginning 2 days after and until trigger administration. To prevent premature luteinizing hormone (LH) surge, a gonadotropin-releasing hormone (GnRH) antagonist (250 μg/day) was administered when the lead follicle size had reached 14 mm in mean diameter. Serum FSH and estrogen levels were monitored throughout the cycle. Oocyte maturation was triggered by 5,000–10,000 IU of human chorionic gonadotropin (hCG; Organon) or 250 μg of recombinant hCG (Serono) or GnRH agonist (leuprolide acetate, 1 mg; Ferring Pharmaceuticals) when at least two follicles had reached the mean diameter of 20–21 mm. Letrozole was discontinued on the day of trigger administration. Transvaginal ultrasound guided oocyte retrieval was performed 35 hours after the trigger. The estradiol (E_2) measurement was repeated 3 days after oocyte retrieval in patients triggered by hCG; if the E_2 level was >250 pg/mL, letrozole was continued approximately 3 to 6 days until the E_2 levels decreased to <50 pg/mL (10). The same procedure was performed for the patients who had a second cycle. All cycles were initiated after breast surgery.

The total gonadotropin and letrozole dose received, number of oocytes retrieved, number of mature and fertilized oocytes and number of embryos frozen after intracytoplasmic sperm injection were assessed and compared between both groups. Follow-up information was collected during return office visits, by phone interview, or by contacting the referring oncologist. Recurrence for breast cancer was defined as the detection of regional tumor, distant metastases, or contralateral invasive breast cancer, and the recurrence rates were compared between groups.

Statistical analysis was performed with the SPSS 15 for Windows package (SPSS, Chicago, IL). Continuous data (presented as mean ± standard deviation) were analyzed using Student's *t*-test and analysis of variance (ANOVA) as appropriate. Variables were complied using visual (histograms, probability plots) and analytic methods (Kolmogorov Simirnov/Shapiro-Wilks test) to determine the normality of distributions. Log rank was used to compare the survival curves (time interval from surgery to chemotherapy) between the groups. Chi-square and, where appropriate, Fischer's exact tests were used to compare proportions of different groups, with $P<.05$ considered statistically significant. For multivariate analyses, a multiple linear regression model was further entered to control for the multiple factors. The

model fit was assessed using appropriate residual and goodness-of-fit statistics.

RESULTS

A total of 157 patients with breast cancer underwent ovarian stimulation, among whom 78 met our inclusion criteria. The baseline patient characteristics are shown in Table 1. Of those women, 61 patients received single cycle stimulation, and 17 patients received two consecutive ovarian stimulation cycles. The two groups were similar with respect to age at diagnosis, body mass index, breast cancer diagnosis, tumor characteristics (tumor size, number of positive lymph nodes, grade, HER-2/neu overexpression, and vascular space invasion) and BRCA status (see Table 1). Multiple factors such as age, FSH levels, antral follicle count, tumor size, lymph node involvement, tumor grade, number of oocytes retrieved, and embryos generated in the first cycle did not influence whether patients chose to do one cycle or two after multivariate analyses.

Statistically significant differences were found in the timing of referral to fertility counseling. The majority of two-cycle patients were referred before surgery (82.3%) compared with only 34.4% of single-cycle patients ($P=.001$).

The majority of patients were triggered by hCG in both groups (12 of 17 vs. 38 of 61; $P=.528$). Although 3 of 17 patients had only oocyte cryopreservation after two consecutive cycles, 11 out of 61 patients had only oocyte cryopreservation in the single-cycle group ($P=.97$). Between each ovarian stimulation cycle, the mean number of oocytes harvested (7.7 ± 5.4 vs. 8.4 ± 9.6 vs. 9.1 ± 5.2) and embryos frozen (3.5 ± 2.6 vs. 3.4 ± 2.6 vs. 3.7 ± 3.1) were not statistically significantly different (Table 2). As expected, for patients receiving two consecutive stimulation cycles, the cumulative mean number of oocytes retrieved (16.1 ± 13.2 vs. 9.1 ± 5.2; $P=.008$) and embryos frozen (6.4 ± 2.9 vs. 3.7 ± 3.1; $P=.019$) were statistically significantly higher compared with those who had only received a single cycle (Table 3).

When the mean time intervals from breast surgery to chemotherapy were considered, no statistically significant difference was seen with 63.7 ± 7.7 compared with 58.0 ± 12.1 days in patients undergoing two cycles versus a single cycle, respectively. Chemotherapy was initiated on average 5.7 days earlier in performing a single cycle compared with performing two cycles, though this difference did not reach statistical significance ($P=.176$) (Fig. 1). Furthermore, the mean time intervals from initial diagnosis to first ovarian stimulation and initial diagnosis to chemotherapy were 36.8 ± 6.4 versus 43.6 ± 13.4 and 81.3 ± 10.2 versus 76.1 ± 10.4 days in patients undergoing two cycles and a single cycle, respectively ($P=.057$; $P=.096$).

In terms of patient follow-up observation after cycle stimulation, the difference in mean follow-up time for single-cycle patients was 56.6 ± 8.4 months versus 67.5 ± 14.4 months for two-cycle patients. The mean follow-up period for both groups was 58.5 ± 13.6 months. Twenty percent (n = 12) of single-cycle patients were lost to follow-up observation; however, the baseline characteristics of these patients were consistent with lower risk of recurrence. Specifically, they all had a tumor size <2 cm with grade 1–2, and only 2 out of 12 patients had lymph node involvement. No breast cancer recurrence was observed in patients undergoing two cycles (0 of 17), compared with two recurrences in single-cycle patients (2 of 49). This was not a statistically significant difference ($P=.548$). One of these recurrences was local, and the other was distant.

TABLE 1

The comparison of patients' demographics and tumor characteristics.

Characteristics	Two cycle (n = 17)	Single cycle (n = 61)	*P* value
Age at diagnosis (y)	35.7 ± 0.4	35.9 ± 0.6	NS
BMI (kg/m^2)	23.1 ± 4.3	22.7 ± 2.7	NS
FSH levels on day 2 (IU/mL)	9.6 ± 3.2	9.3 ± 3.2	NS
Antral follicle count	10.9 ± 9.8	8.2 ± 4.7	NS
Tumor characteristics			
Histologic grade			
Grade 1–2	16/17 (94.2%)	58/61 (95.1%)	NS
Grade 3	1/17 (5.8%)	3/61 (4.9%)	NS
Nodal status			
Negative	9/17 (64.8%)	39/61 (63.4%)	NS
Positive	6/17 (35.2%)	22/61 (36.6%)	NS
Lymph vascular invasion (+)	3/17 (17.6%)	15/61 (24.5)	NS
Tumor size (cm)	1.8 ± 0.9	1.7 ± 0.1	NS
ER (+)	11/17 (64.7%)	35/61 (69.3%)	NS
PR (+)	11/17 (64.7%)	33/61 (65.3%)	NS
HER-2/neu (+)	4/17 (23.5%)	13/61 (22.4%)	NS
BRCA (+)	3/17 (17.6%)	9/61 (12.2%)	NS
Family history of cancer (+)	10/17 (58.8%)	34/61 (65.3%)	NS

Note: Values for continuous variables are mean ± standard deviation. Values for categorical variables are number/total number of cases (%). $P<.05$ was considered statistically significant. BMI = body mass index; ER = estrogen receptor; FSH = follicle-stimulating hormone; NS = not statistically significant; PR = progesterone receptor.

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DISCUSSION

The main principle of fertility preservation counseling should be to recommend and use the most successful and the least experimental approach for young women who are diagnosed with cancer (11). However, concerns related to estrogen exposure and the potential delay in the initiation of chemotherapy limit the access of women diagnosed with breast cancer to established procedures requiring ovarian stimulation, namely, embryo cryopreservation and oocyte cryopreservation. We previously described that breast cancer recurrence rates are not increased after ovarian stimulation with the letrozole–gonadotropin protocol (12). Our present study investigated the feasibility of performing two consecutive cycles before breast cancer treatment, and no recurrence in breast cancer was found in the breast cancer patients who underwent two consecutive cycles of ovarian stimulation during 67.5 ± 14.4 months of follow-up evaluation. Nonetheless, future studies should be planned to compare the recurrence rates between single cycle and multiple cycles, including a longer time period of follow-up observation and a larger number of patients.

Women with breast cancer typically have an interval of 6 to 8 weeks between surgery and the initiation of adjuvant chemotherapy and several studies demonstrated that chemotherapy could be delayed up to 12 weeks after surgery in patients with early stage breast cancer (7, 8). In the present study, the time interval from breast surgery to

TABLE 2

Fertility preservation cycle outcomes of patients with breast cancer before initiation of treatment.

Outcome	Single cycle (n = 61)	Two cycles (n = 17)		*P* value
		First cycle	Second cycle	
Total letrozole dose (mg)	50.3 ± 9.9	53.2 ± 13.4	51.1 ± 10.0	NS
Total rFSH dose (IU)	2320.9 ± 889.9	2369.5 ± 833.4	2522.7 ± 931.7	NS
Peak E_2 levels (pg/mL)	535.0 ± 448.0	374.0 ± 196.0	337.5 ± 185.1	NS
Oocytes (n)	9.1 ± 5.2	7.7 ± 5.4	8.4 ± 9.6	NS
Mature oocytes (n)	6.2 ± 3.0	5.1 ± 3.1	5.5 ± 4.7	NS
Inseminated oocytes (n)	6.0 ± 3.9	4.9 ± 3.8	4.9 ± 3.0	NS
Fertilized oocytes (n)	5.4 ± 2.3	4.4 ± 2.8	4.1 ± 3.7	NS
Embryos (n)	3.7 ± 3.1	3.5 ± 2.6	3.4 ± 2.6	NS

Note: Results were given as mean ± standard deviation. *P*< .05 was considered statistically significant. E_2 = estradiol; NS = not statistically significant; rFSH = recombinant follicle-stimulating hormone.

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chemotherapy in patients undergoing two consecutive cycles of ovarian stimulation was 63.7 ± 7.7 days. Although this time period was longer when compared with performing single cycle (on average 5.7 days), the difference was not statistically significant and may not be considered meaningful clinically relevant. If patients are referred as early as possible after initial diagnosis, fertility preservation cycles can be initiated sooner, and the time interval from surgery to chemotherapy is reduced (13). In the present study, 82.3% of patients having two cycles were referred before breast cancer surgery whereas only 34.4% of patients having single cycle were referred before surgery. Thus, early referral can be one of the major factors in determining the number of ovarian stimulation cycles performed.

We recently reported the importance of early referral (13). In that study, 93 patients with breast cancer were divided into two groups (before or after breast surgery) according to their referral time to reproductive specialist. Although 9 of 35 patients who were in the presurgery group had a chance to undergo two consecutive ovarian stimulation cycles, only one of 58 patients in the postsurgery group had a chance to undergo two consecutive cycles. At the end of that study, we had shown that the patients in the presurgery group initiated chemotherapy on average 24 days earlier compared with patients in the postsurgery group.

The American Society of Clinical Oncology (14) and the American Society for Reproductive Medicine recommend attention to the impact of cancer treatments on infertility. Despite the fact that 57% of patients reported substantial concern at diagnosis about seeing an infertility specialist (15), recent studies have indicated that still less than half of physicians routinely refer their reproductive-aged patients with cancer to a reproductive specialist (16). As well as the reproductive specialist, breast surgeons and medical oncologist should be involved in the management of these patients to increase the likelihood of success rate of fertility preservation.

A relatively small sample size and short follow-up time may be the limitations of this study. Additional studies are necessary, including those with a larger number of patients and longer follow-up periods, to further evaluate these findings.

TABLE 3

Comparison of fertility preservation cycle outcomes of patients with breast cancer after performing two cycles.

Outcome	Single cycle (n = 61)	Two cycles (n = 17)	*P* value
Oocytes (n)	9.1 ± 5.2	16.1 ± 13.2	.008
Mature oocytes (n)	6.2 ± 3.0	10.3 ± 7.7	.004
Inseminated oocytes (n)	6.0 ± 3.9	9.8 ± 5.5	.002
Fertilized oocytes (n)	5.4 ± 2.3	7.4 ± 3.9	.040
Embryos (n)	3.7 ± 3.1	6.4 ± 2.9	.019

Note: Results were given as mean ± standard deviation. *P*< .05 was considered statistically significant.

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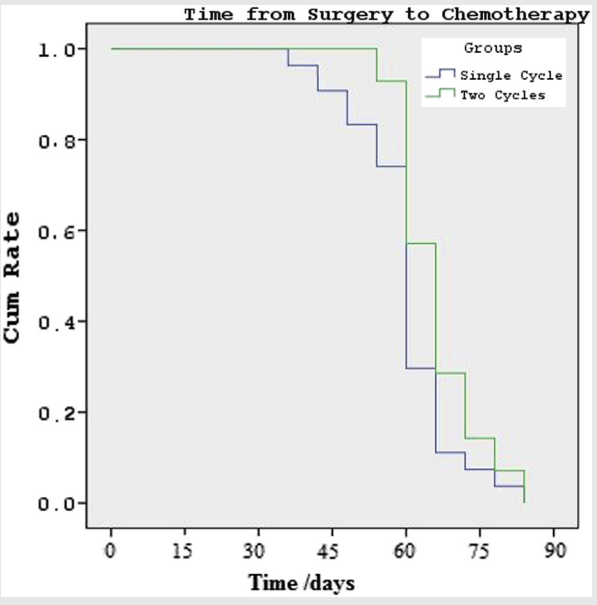
FIGURE 1



Time interval from surgery to chemotherapy in patients undergoing two cycles versus single cycle. After log rank test, no difference was found between two groups (*P*=.176).

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Our study suggests that performing two consecutive cycles of ovarian stimulation with letrozole and gonadotropin protocol for fertility preservation before chemotherapy is feasible and can provide a larger number of oocytes and embryos for cryopreservation. Early referral may provide additional time for women with breast cancer to undergo two consecutive cycles without a significant delay in the initiation of chemotherapy.

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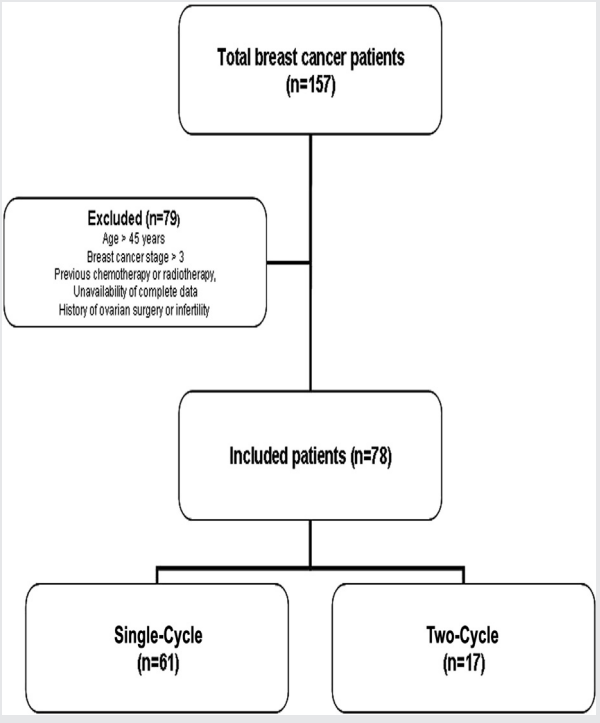
REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10–29.
2. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 2011;61:212–36.
3. Valdivieso M, Kujawa AM, Jones T, Baker LH. Cancer survivors in the United States: a review of the literature and a call to action. Int J Med Sci 2012;9:163–73.
4. Rodriguez-Wallberg KA, Oktay K. Options on fertility preservation in female cancer patients. Cancer Treat Rev 2012;38:354–61.
5. Checa Vizcaino MA, Corchado AR, Cuadri ME, Comadran MG, Brassesco M, Carreras R. The effects of letrozole on ovarian stimulation for fertility preservation in cancer-affected women. Reprod Biomed Online 2012;24:606–10.
6. Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, Bang H. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab 2006;91:3885–90.
7. Cold S, During M, Ewertz M, Knoop A, Moller S. Does timing of adjuvant chemotherapy influence the prognosis after early breast cancer? Results of the Danish Breast Cancer Cooperative Group (DBCG). Br J Cancer 2005;93:627–32.
8. Lohrisch C, Paltiel C, Gelmon K, Speers C, Taylor S, Barnett J, Olivotto IA. Impact on survival of time from definitive surgery to initiation of adjuvant chemotherapy for early-stage breast cancer. J Clin Oncol 2006;24:4888–94.
9. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. Fertil Steril 2013;100:492–9.
10. Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol 2005;23:4347–53.
11. Bedoschi G, Oktay K. Current approach to fertility preservation by embryo cryopreservation. Fertil Steril 2013;99:1496–502.
12. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol 2008;26:2630–5.
13. Lee S, Ozkavukcu S, Heytens E, Moy F, Oktay K. Value of early referral to fertility preservation in young women with breast cancer. J Clin Oncol 2010;28:4683–6.
14. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. J Clin Oncol 2013;31:2500–10.
15. Duffy CM, Allen SM, Clark MA. Discussions regarding reproductive health for young women with breast cancer undergoing chemotherapy. J Clin Oncol 2005;23:766–73.
16. Quinn GP, Vadaparampil ST, Lee JH, Jacobsen PB, Bepler G, Lancaster J, et al. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. J Clin Oncol 2009;27:5952–7.

SUPPLEMENTAL FIGURE 1



Configuration of all breast cancer patients in this study.

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