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Ovarian Stimulation in Patients With Cancer: Impact of Letrozole and BRCA Mutations on Fertility Preservation Cycle Outcomes

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Abstract

Background: Aromatase inhibitors (AI) have been introduced to reduce estrogen exposure in women with estrogen-sensitive cancer undergoing ovarian stimulation for oocyte/embryo cryopreservation. There have been questions regarding whether the addition of AI and the presence of BRCA mutations affect cycle outcomes. We sought to determine the impact of letrozole and BRCA mutations on fertility preservation (FP) cycle outcomes of patients undergoing ovarian stimulation with an antagonist protocol. **Methods:** The data were generated by the secondary analysis of a prospective database of all females diagnosed with cancer who underwent embryo or oocyte cryopreservation for FP. The final analysis included 145 patients stimulated with an antagonist protocol either using letrozole combined with recombinant follicle-stimulating hormone (rFSH; LF, n = 118) or rFSH alone (FA, n = 24). **Results:** The mean number of total (15.6 [7.9] vs 10.2 [7.8]; $P = .004$) and mature oocytes (10.4 [5.1] vs 7.8 [3.5]; $P = .044$) and embryos frozen (7.7 [5.3] vs 5.3 [2.7]; $P = .043$) were significantly higher after LF stimulation versus FA. In the LF group, women with BRCA mutations produced significantly fewer oocytes (11.0 [8.0] vs 16.4 [7.7], $P = .015$) and embryos (5.1 [4.4] vs 8.2 [4.7], $P = .013$), compared to those who were mutation negative. After adjusting for age, body mass index, baseline FSH level, and BRCA status, LF protocol still resulted in higher number of total oocytes (95% confidence interval [CI]: 1.9 to 3.6; $P = .002$) mature oocyte (95% CI: 0.3 to 1.4; $P = .028$), and embryo yield (95% CI: 0.7 to 1.4; $P = .015$). **Conclusion:** In women with cancer undergoing FP, letrozole appears to enhance response to ovarian stimulation while the presence of BRCA mutations is associated with lower oocyte and embryo yield.

Keywords

BRCA, cancer, embryo cryopreservation, letrozole, ovarian response

Introduction

The last few decades witnessed tremendous advances in the early diagnosis and treatment of cancer, enabling higher survival rates and a growing population of young cancer survivors who are left with the after effects of cancer treatments.[1,2] One of the most significant effects of cancer treatments on the quality of life is the damage to ovarian function and future reproductive potential.[3] As a result of the growing emphasis on the quality of life issues of patients with cancer, fertility preservation has gained preeminence in the care of young survivors.[4]

The selection of a fertility preservation option in patients with cancer may be influenced by the patient's sexual maturity and age, presence or absence of a partner, the time available before the initiation of chemotherapy, and the patient or couple's family building goals.[5,6] Embryo and oocyte cryopreservation are considered established fertility preservation techniques and are generally first-line approaches when there is sufficient time for ovarian stimulation.[7,8] However, the exposure to high levels of estradiol (E2) during stimulation is generally considered contraindicated in patients with breast cancer.[9] To reduce the potential risks of increased estrogen exposure from conventional ovarian stimulation protocols, we developed alternative approaches such as the

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coadministration of aromatase inhibitor letrozole.[10] In both short- and long-term studies, we have shown that the recurrence risk is not increased in women with breast cancer who had undergone ovarian stimulation with letrozole and recombinant follicle-stimulating hormone (rFSH).[11-13] Moreover, Letrozole and recombinant FSH (LF) protocol resulted in the preservation of fertility in at least half of the women with breast cancer who underwent embryo cryopreservation at a mean age of 36, with 45% live birth per embryo transfer rate.[14] Despite the encouraging data on the success of the LF protocol in women with breast cancer, there has been a discussion of its efficacy compared to the standard protocols. Letrozole is a selective and highly potent third-generation aromatase inhibitor that reduces plasma concentrations of estrogens by inhibiting the conversion of androgens to estrogens.[15,16] This acute decline in circulating estrogen levels results in the release of FSH from the pituitary by breaking negative feedback loop on the hypothalamic–pituitary axis, which in turn causes ovarian stimulation.[17] Moreover, by blocking the conversion from androgens to estrogens, aromatase inhibitors increase intraovarian androgens, which have been shown to augment early follicular development.[18,19] Based on these prior observations and studies, we therefore hypothesized that the addition of letrozole to a standard short antagonist protocol would increase the oocyte yield. Although we previously showed that the LF protocol resulted in comparable outcomes in patients with cancer relative to those undergoing In vitro fertilization (IVF) with standard protocols for tubal factor infertility,[20] no such comparison exists within the cancer population. We have also shown in a prior small study that the presence of BRCA mutations is associated with lower ovarian response among women undergoing ovarian stimulation with a letrozole protocol, compared to those who were BRCA mutation negative or untested. However, a larger study has been awaited to confirm the latter findings.

Therefore, in this study, our primary aim was to compare the efficacy of ovarian stimulation using letrozole combined with rFSH to that of using rFSH alone in patients diagnosed with cancer who desired fertility preservation. Our secondary aim was to test the impact of BRCA mutations on cycle outcomes after stimulation with the LF protocol.

Materials and Methods

Patients

The data were generated by the secondary analysis of a prospectively maintained database of all women diagnosed with cancer who underwent assisted reproductive technology treatments for fertility preservation at our institution. The study protocol was approved by the institutional review board at New York Medical College. We excluded patients >40 years of age and those who were infertile, had a history of ovarian surgery, or prior exposure to chemotherapy or radiotherapy. Women with breast cancer were stimulated with LF protocol because of the hormonal sensitivity of their cancers. Breast cancers were considered hormonally sensitive regardless of the tumor receptor status, as high estrogen levels can have nonreceptor-dependent actions on the tumor tissue.[21] The decision for BRCA testing was made by the referring medical oncologists based on established criteria and independent of this study. During the period of our data collection, the recommendation was to test women under age 50 years with triple-negative breast cancer and women with a family history of early-onset breast cancer or ovarian cancer.[22] Women with nonhormone-dependent cancers such as myelodysplastic syndrome, leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, cervical cancer, brain tumors, retinoblastoma, synovial sarcoma, thyroid cancer were stimulated with an rFSH alone (FA) protocol (Figure 1).

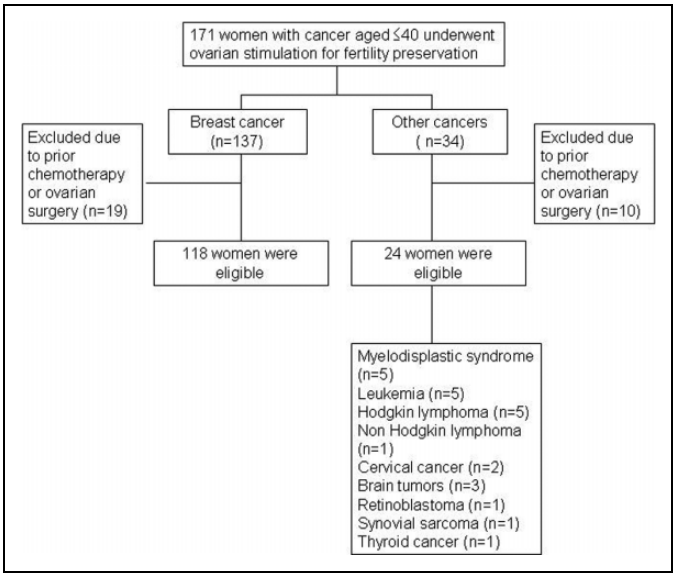

Figure 1. Study diagram.

Ovarian Stimulation Protocols

In patients with breast cancer, 5 mg/d letrozole (Femara; Novartis, East Hanover, New Jersey) was initiated on cycle day 2 or 3. Daily injections of rFSH (Follistim; Merck Sharp and Dohme, Whitehouse Station, New Jersey) were added 2 days later. The starting dose of rFSH was 150 to 300 IU based on the patients' ovarian reserve, age, and body mass index. The dose was adjusted during the cycle, if necessary. To prevent a premature luteinizing hormone (LH) surge, patients were given 0.25 mg/d of a gonadotropin-releasing hormone (GnRH) antagonist (Ganirelix; Organon, West Orange, New Jersey) when the lead follicle size reached $\geq$13 mm mean diameter; this dose was continued until the trigger day. When a minimum of 2 follicles reached at least 20 mm in diameter,[20] oocyte maturation was triggered with either human chorionic gonadotropin (hCG; Ovidrel; EMD Serono, Rockland, Massachusetts) or 1 mg leuprolide acetate (Lupron; Ferring Pharmaceuticals, Parsippany, New Jersey). Letrozole was discontinued on the day of the trigger. Transvaginal ultrasound-guided oocyte

retrieval was performed 35 hours after the trigger. The E2 measurement was repeated 3 days after oocyte retrieval in patients triggered with hCG; if the E2 level was >250 pg/mL, letrozole was continued approximately 3 to 6 days until the E2 levels decreased to <50 pg/mL.[23] For those patients who are not undergoing oocyte cryopreservation, all mature oocytes were fertilized by intracytoplasmic sperm injection for subsequent embryo freezing.

In women with nonbreast cancers, a similar antagonist protocol was utilized. Ovarian stimulation was started on cycle day 2 or 3 using 150 to 300 IU/d rFSH alone. The criteria for starting GnRH antagonist administration to prevent a premature LH surge were the same as in the other group. The criterion for trigger administration was the presence of at least 2 follicles $\geq$18 mm in mean diameter. Of note, the only difference between the 2 protocols other than the utility of letrozole was the trigger criteria. Because our earlier work showed that follicles develop antral space earlier when stimulated with letrozole and the optimal maturity is reached at approximately 2 mm larger diameter than when using standard protocols,[20] we do trigger when lead follicles reach at least 20 mm in mean diameter in letrozole cycles.

Because some patients underwent 2 consecutive ovarian stimulation cycles, only the first cycle attempt was included in the analysis to avoid dependence among observations. The primary outcome measures were the total number of oocytes and embryos obtained per cycle.

Statistical Analysis

Statistical analysis was performed by an expert biostatistician (FM) using the Statistical Package for the Social Sciences (release 15.0; SPSS Inc, Chicago, Illinois). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk test) to determine whether they were normally distributed. Although normally distributed continuous data were analyzed by Student *t* test (age, baseline FSH and estrogen levels, estrogen levels on trigger day, number of oocytes retrieved, number of embryos cryopreserved), Mann-Whitney *U* test was used for nonnormally distributed data (starting and total rFSH dose and fertilization rate). A multivariate regression analysis was performed to adjust for the following potential confounders: age, body mass index, baseline FSH level, BRCA status. The χ^2 was used to compare proportions where appropriate. A *P* value $\leq$.05 was considered statistically significant. Data were presented as mean (standard deviation).

Results

One hundred and seventy-one women underwent ovarian stimulation for fertility preservation before cancer treatments. After the exclusions, there remained 118 women in the LF and 24 women in the FA group. In the LF group, 21 had BRCA mutations, 12 were not tested because they were not found at high risk for carrying BRCA mutations based on clinical criteria, and the remaining tested negative for known BRCA mutations. In the FA group, 16 women were diagnosed with hematological cancers, while the remaining had cervical cancer (n = 2), brain tumors (n = 3), retinoblastoma (n = 1), synovial sarcoma (n = 1), or thyroid cancer (n = 1).

Table 1. Comparison of the Study Group Characteristics.

Variables	LF (n = 118)	FA (n = 24)	*P* Value
Age, years	33.1 (3.1)	30.8 (5.9)	.085
BMI, kg/m^2	22.1 (3.5)	24.6 (6.8)	.072
Baseline FSH levels, IU/mL	8.4 (3.8)	8.1 (3.4)	.788
Baseline E2 levels, pg/mL	43.6 (26.9)	53.8 (50.7)	.215
Starting FSH dose, IU	246.0 (77.4)	254.3 (69.7)	.612
Total FSH dose, IU	1956.6 (940.5)	2216.6 (1159.4)	.234
Total letrozole dose, mg	47.9 (11.3)	N/A	
Ovarian stimulation length, days	10.6 (1.7)	10.0 (1.9)	.157
E2 on the trigger day, pg/mL	665.2 (569.1)	1254.9 (586.0)	<.001
Number of patients triggered with GnRHa, (%)[a]	39/118 (33%)	9/24 (37%)	.674

Abbreviations: BMI, body mass index; FA, rFSH alone; FSH, follicle-stimulating hormone; GnRHa, gonadotropin-releasing hormone agonist; SD, standard deviation.
[a]Remaining patients were triggered with human chorionic gonadotropin (hCG). Results were given as mean (SD). A *P* value $\leq$.05 was considered statistically significant.

The women who were stimulated with LF showed a trend for being older compared to those being stimulated with FA (33.1 [3.1] vs 30.8 [5.9] years, respectively; $P = .085$). No significant differences were found in baseline E2 or FSH levels between the LF and FA groups. A comparison of the demographic characteristics of the study groups is shown in Table 1.

Embryo cryopreservation occurred in 95 women in the LF and 18 women in the FA group with the remaining undergoing oocyte cryopreservation in both groups. Although the duration of ovarian stimulation and the total dose of FSH were similar, peak E2 levels were significantly lower in the LF group (665.2 [569.1] vs 1254.9 [586.0] pg/mL; $P < .001$). The mean number of oocytes retrieved (15.6 [7.9] vs 10.2 [7.8], respectively; $P = .004$), mature oocytes (10.4 [5.1] vs 7.8 [3.5], respectively; $P = .044$), and embryos frozen (7.7 [5.3] vs 5.3 [2.7], respectively; $P = .043$) were significantly higher in the LF group.

Since BRCA mutations have been found to be associated with lower ovarian reserve in several prior studies including ours,[24,25] we next tested the impact of BRCA mutations on ovarian response in the LF group. Our analysis showed that those with BRCA mutations produced fewer oocytes (16.4 [7.7] vs 11.0 [8.0], $P = .015$) and embryos (8.2 [4.7] vs 5.1 [4.4], $P = .013$) compared to those who were BRCA negative or untested. After adjusting for age and BMI, these differences became more prominent with marginally lower fertilization rates in women with BRCA mutations (Table 2), confirming and extending our observations in a larger cohort of patients.

Table 2. Impact of BRCA Mutations on Response to Ovarian Stimulation With Letrozole-FSH Protocol.

Variables and Outcomes	BRCA negative or untested (n = 97)	BRCA positive (n = 21)	*P* Value	Adjusted *P* Value[a] (95% CI)
Age	33.2 (3.2)	32.7 (2.4)	.680	
BMI, kg/m^2	22.1 (3.1)	23.5 (5.2)	.695	
No. of total oocytes	16.4 (7.7)	11.0 (8.0)	.015	.002 (−10.6 to −2.5)
No. of mature oocytes	10.6 (5.1)	7.4 (5.7)	.047	.008 (−7.2 to −1.1)
Fertilization rate, %	79.3 (16.4)	74.0 (24.8)	.277	.053 (−20.1 to 0.2)
No. of embryos frozen	8.2 (4.7)	5.1 (4.4)	.013	.003 (−7.1 to −1.5)

Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation.
[a]Adjusted for age and body mass index. Starting and total gonadotropin use did not differ between the groups. Results were given as mean (SD). A *P* value ≤.05 was considered statistically significant.

Table 3. Comparison of Letrozole-FSH to FSH-only Protocol in Fertility Preservation Patients.[a]

Cycle outcomes	LF (n = 118)	FA (n = 24)	*P* Value	Adjusted *P* Value[b] (95% CI)
No. of total oocytes	15.6 (7.9)	10.2 (7.8)	.004	.002 (1.9 to 3.6)
No. of mature oocytes	10.4 (5.1)	7.8 (3.5)	.044	.028 (0.3 to 1.4)
Fertilization rate, %	78.0 (18.6)	87.5 (14.6)	.107	.489 (−14.4 to 6.9)
No. of embryos frozen[c]	7.7 (5.3)	5.3 (2.7)	.043	.015 (0.7 to 1.4)

Abbreviations: CI, confidence interval; FA, rFSH alone; FSH, follicle-stimulating hormone, SD, standard deviation.
[a]Results were given as mean (SD). A *P* value ≤.05 was considered statistically significant.
[b]Adjusted for age, body mass index, baseline follicle-stimulating hormone (FSH) level and BRCA status.
[c]Ninety-five women in the LF and 18 in the FA group underwent embryo cryopreservation while others underwent oocyte cryopreservation.

Given its confirmed association with lower response to ovarian stimulation, we therefore included BRCA mutation status in our final model for logistic regression. After adjusting for age, BMI, baseline FSH level, and BRCA mutation status, LF protocol still resulted in higher number of total oocytes (95% CI: 1.9-3.6; $P = .002$), mature oocyte (95% CI: 0.3-1.4; $P = .028$), and embryo yield (95% CI: 0.7-1.4; $P = .015$) compared to FA protocol (Table 3).

Discussion

In this study, we compared a short antagonist protocol with letrozole (LF) to a similar protocol without the co-treatment of letrozole (FA) in patients with cancer and found that LF protocol resulted in higher number of oocytes and embryos obtained. After adjusting for various confounders including the BRCA mutation status, total number of retrieved oocytes and obtained embryos were still higher in the LF group.

A biological rationale exists for the possible augmentative benefit of aromatase inhibitors in ovarian stimulation. Previous studies demonstrated that ovarian stimulation with letrozole increases intraovarian androgen concentrations and improves response to ovarian stimulation.[26,27] Androgens serve as a substrate for E2 production and promote the growth of small follicles and the proliferation of granulosa and theca cells to augment follicular sensitivity to FSH.[26,27] Intraovarian androgens have also been shown to have a profound supportive impact on mouse preantral follicles in vitro.[28] Furthermore, aromatase inhibitor treatment augments preantral and small antral follicle growth in vitro.[18] In the present study, administration of letrozole from cycle day 2 may have resulted in a more supportive androgenic environment for small follicles by increasing their gonadotropin sensitivity and reducing the tendency for atresia.

To our knowledge, our study is the only large study comparing an antagonist protocol with or without letrozole cotreatment in a cancer population. In a pilot study comparing ovarian stimulation with aromatase inhibitors in patients with breast cancer (n = 9) to standard ovarian stimulation in nonhormone-dependent patients with cancer (n = 10), the total number of oocytes retrieved was not significantly different between the groups.[29] However, the small number of patients did not provide the statistical power to compare the cycle outcomes.

The patients in the 2 study arms were treated with identical short antagonist protocols with the difference being the use of letrozole in women with breast cancer. In addition, the trigger was performed at a 2 mm larger end point based on our earlier work.[20] The length of ovarian stimulation with the LF protocol is similar to standard protocols and the difference in the follicle size at the time of trigger appears to be due to the fact that letrozole results in the earlier formation of antral space.[20,23] Hence when we use a trigger criteria with larger follicle size with letrozole, the physiological state of the lead follicles appears to be more similar to those stimulated with standard protocols. In fact, we have shown that when trigger criteria for standard protocols are used for letrozole cycles, this results in a higher immaturity and lower fertilization rates.[20] This fact may explain why some ovulation induction studies, which used identical trigger criteria for all groups, may have observed lower success with letrozole.[30]

Our study has several strengths and weaknesses. Although we compared the letrozole protocol in patients with breast cancer to standard protocols in tubal factor infertility patients in previous studies, this study accomplished the same

comparison within a cancer population. Because there could be differences between the infertile women and women with cancer in terms of ovarian response to stimulation, our study presents a more valid analysis of the addition of letrozole to an antagonist protocol in fertility preservation. An additional strength of this study is that all cycles were managed by a single investigator (KO), ensuring uniformity.

In theory, the ideal design would be to randomize patients with breast cancer to receiving a letrozole protocol versus a nonletrozole protocol. Unfortunately, due to the hormone responsiveness of even the ER-negative breast cancers, this would not be practical. For the same reason, a randomized study among Estrogen receptor (ER)-negative patients would also be considered unethical.[21]

Instead, our study was a secondary analysis of a prospectively collected database. We could not randomize women with breast cancer to protocols with and without letrozole, as the utility of standard protocols is considered contraindicated in women with estrogen-sensitive cancers. For the latter reason, FA protocol was tested in a group predominantly with hematological cancers. Although it could be questioned that the differences in cancer types between the 2 groups could also differentially affect the response to ovarian stimulation, claims have been made for both women with breast cancer[24,31] and women with hematological cancers[32] that ovarian response may be lower than in healthy women. In fact, in our study, women with breast cancer were expected to have lower response, given the confirmed association of BRCA mutations with lower ovarian response. Contrary to these expectations, we found higher oocyte and embryo yield with letrozole both before and after the adjustment for BRCA mutation status.

We previously showed that controlled ovarian stimulation in patients with BRCA mutations may result in lower oocyte yield[24,25] and a number of prospective studies supported that ovarian reserve is diminished in women with BRCA mutations.[33-36] This includes a recent large prospective study which found serum Anti-Müllerian hormone (AMH) levels to be lower in women with BRCA mutations.[37] In translational studies involving both mouse and human, we also showed that the mechanism of reduced ovarian reserve in BRCA mutation carriers is via DNA double-strand break (DSB) repair deficiency, as BRCA genes play important roles in the Ataxia telangiectasia mutated (ATM)-mediated DNA DSB repair pathway.[25,38] Lower DNA repair efficiency in the oocytes of women with BRCA mutations seems to lead increased accumulation of lethal DNA DSBs with age, resulting in earlier loss of oocyte reserve via apoptotic death.[25,38] Independent studies in rats and monkeys were also supportive of these mechanistic findings.[39,40] Finally, we have recently shown in a controlled study that primordial follicle loss and oocyte DNA double strand break accumulation are accelerated in the ovaries of women with BRCA germline mutations.[41] In the current study, another novel finding was a trend for lower fertilization rates in women with BRCA mutation, which is in support of our previous data and hypothesis that DNA repair is involved maintaining oocyte quality. Further prospective studies will be needed to determine if women with BRCA mutations have impaired oocyte quality.

In a retrospective study by Shapira et al,[42] it was claimed that women with BRCA mutations show normal ovarian response to controlled ovarian stimulation for in vitro fertilization. In addition to being a retrospective chart review, the study was underpowered to control for many confounders that existed. Despite the biological differences, the study considered BRCA1 and BRCA2 mutation carriers, affected and unaffected, together. The study groups were highly heterogeneous and included those undergoing fertility preservation, preimplantation genetic diagnosis to eliminate inheritance of BRCA mutations, and those undergoing IVF for male and female factor infertility. The possible elimination of severely affected patients with family history and with earlier onset of cancers due to early removal of ovaries or cancer diagnosis and treatment was not taken into account. Furthermore, there was lack of uniformity for ovarian stimulation protocols as well (agonist vs antagonist protocols, rFSH vs human menopausal gonadotropins, tamoxifen vs nontamoxifen). Finally, the diminished ovarian reserve may become clinically more significant during later reproductive ages as earlier research suggested.[25] However in the article by Shapira et al,[41] the mean age of all carriers was 31 years, which may also make it difficult to observe small differences, especially in a retrospective and not well-controlled design.

In conclusion, LF protocol is an effective option in patients with breast cancer undergoing fertility preservation. Although the addition of letrozole appears to enhance oocyte and embryo yield, the response to ovarian stimulation is lower in women with BRCA mutations. Long-term studies should evaluate whether letrozole supplementation enhances fertility preservation success and that ovarian stimulation with a letrozole-gonadotropin protocol should be utilized for all patients with cancer who desire fertility preservation via oocyte or embryo cryopreservation, regardless of the estrogen sensitivity of their tumors.

Declaration of Conflicting Interests

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