Ovarian stimulation and fertility preservation with the use of aromatase inhibitors in women with breast cancer

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Breast cancer is the most common malignancy diagnosed in women in the United States. Many breast cancer survivors are concerned that cancer treatment will compromise their reproductive potential. Despite this concern, most women receive limited information addressing preservation of fertility before initiating adjuvant chemotherapy. Historically, the supraphysiologic levels of estrogens associated with ovarian stimulation have precluded the use of assisted reproductive technologies in the presence of breast cancer. In an effort to mitigate the potential effects of elevated estrogen levels during ovulation induction, we developed a novel ovarian stimulation protocol for women with breast cancer, with the use of aromatase inhibitors. Our studies suggest that in the short term, aromatase inhibitors plus gonadotropins are safe and effective agents for ovarian stimulation in fertility preservation cycles. In this review, we outline the data supporting the use of aromatase inhibitors for ovarian hyperstimulation in women with breast cancer before initiating adjuvant chemotherapy. (Fertil Steril® 2012;98:1363–9. ©2012 by American Society for Reproductive Medicine.)

Key Words: Aromatase inhibitors, breast cancer, fertility preservation, GnRH agonist, letrozole

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Breast cancer is the most common malignancy diagnosed in women in the United States. In 2012, an estimated 230,000 new cases of invasive breast cancer are expected to be diagnosed in American women, whose lifetime risk of developing the disease is one in eight (1). Early detection and improvements in screening have increased the number of premenopausal women diagnosed with breast cancer, while advances in treatment options have contributed to declining breast cancer mortality rates (2). Recent estimates suggest that more than 2 million breast cancer survivors currently live in the United States, comprising the largest constituent of all female cancer survivors (3). With increasing numbers of breast cancer survivors, quality of life issues continue to gain prominence, particularly fertility preservation (4). Many breast cancer survivors are fearful that their history of cancer or its treatment will compromise their fertility. In a novel web-based survey investigating the effects of breast cancer and its treatment options among young breast cancer survivors, 57% of more than 600 respondents reported significant concerns regarding compromised reproductive potential as result of their life-saving treatment, and 29% reported that these concerns guided their treatment decisions (5). Despite these concerns, most reported that they received little information from their oncologists addressing preservation of fertility (5). As a result, the American Society of Clinical Oncology and the American College of Obstetricians and Gynecologists have released guidelines encouraging physicians to refer their cancer patients to fertility specialists before the initiation of treatment (6–8).

Embryo cryopreservation is currently the most established technique for fertility preservation (9). According to most recent data published by the Society for Assisted Reproductive Technologies, the current live-birth rate per transfer of frozen-thawed embryos is 38.7% in U.S. women <35 years old (10). Although data on ooycte cryopreservation is promising, it remains investigational at this time (11, 12). Because

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women with breast cancer typically have a window of ~6 weeks between surgery and the initiation of adjuvant chemotherapy, it is feasible to undergo controlled ovarian hyperstimulation (COH) (13, 14). During this process, exogenous gonadotropins are used alone or in combination to stimulate the growth and maturation of multiple oocytes, often resulting in supraphysiologic levels of estradiol (E_2) (15). Compared with spontaneous cycles, the E_2 levels in stimulated cycles are substantially higher (16). Given the growing body of evidence linking prolonged estrogen exposure and breast cancer, most oncologists and breast cancer patients are reticent to pursue assisted reproductive technologies (ART), fearing that the high estrogenic state can promote cancer growth or recurrence (17). As a result, breast cancer patients are usually offered natural-cycle IVF, which we have found to result in a single embryo in ~60% of the preservation cycles (18). Furthermore, breast cancer survivors may even question the safety of future pregnancy. Several population-based studies have failed to identify a detrimental association between pregnancy and risk of recurrence or mortality from breast cancer (19–21). A recent meta-analysis of 14 studies, which included more than 18,000 control and 1,200 case subjects, reported similar relapse-free survival rates between women who conceived and women who chose not to conceive (22). This further highlights the importance of providing breast cancer survivors with accurate information to make informed decisions regarding future fertility.

In an effort to mitigate the potential effects of elevated estrogen levels during COH for fertility preservation, we developed a novel ovarian stimulation protocol with the use of aromatase inhibitors (AI) (23–25). Aromatase is part of the cytochrome P-450 enzyme complex that catalyzes the rate-limiting step in the biosynthesis of endogenous estrogens, specifically the conversion from androstenedione and testosterone to estrone and E_2, respectively (26). Aromatase activity is present in tissues throughout the body, including the ovaries, breast, brain, liver, and adipose tissue. The predominant source of endogenous estrogens in premenopausal women is the ovaries, and in postmenopausal women it is adipose tissue (27). Because aromatase catalyzes the final step in the production of endogenous estrogens, cancer researchers have targeted it for selective inhibition in hopes of reducing the levels of circulating estrogens. Several landmark studies have demonstrated that AIs significantly reduce the risk of recurrence in postmenopausal women with hormone receptor–positive breast cancer (28–30). This dramatic effect has been attributed to the profound estrogen deprivation induced by third-generation AIs (27). The short-term use of AIs, and subsequent reduction in circulating estrogen, should release the hypothalamic-pituitary axis from the central effects of estrogen-mediated negative feedback. As a result, FSH is increased, which is ultimately responsible for folliculogensis (31).

Given the initial studies reporting the promising use of AIs for ovulation induction without significantly raising E_2 levels, we sought to use AIs in fertility preservation cycles for women with breast cancer (32–34). In a prospective cohort study of 60 women aged 24–43 years with breast cancer, 29 women elected to undergo IVF before adjuvant chemotherapy and the remaining 31 elected not to undergo IVF and served as the control group. Of the 29 women who elected to proceed to IVF, they were assigned to one of three arms based on patient self-selection and/or physician assignment. The three groups consisted of tamoxifen plus FSH, letrozole plus FSH, or tamoxifen alone. Seven women received 60 mg tamoxifen (Aztra Zeneca) plus 150 IU FSH (Gonal-F, Serono; or Follistim, Organon) daily from cycle day 2 or 3 until hCG administration. Eleven women received 5 mg letrozole daily starting on cycle day 2 or 3 and, after 2 days, 150 IU/d FSH until hCG administration. If E_2 levels remained elevated after oocyte retrieval, letrozole was restarted. The final group of 12 women received 60 mg tamoxifen daily until hCG administration. Compared with the tamoxifen-alone group, the tamoxifen plus FSH and the letrozole plus FSH groups had a significantly greater number of follicles ($P<.0001$), mature occytes ($P<.001$), and embryos ($P<.001$). On the other hand, the peak E_2 levels were significantly lower in the tamoxifen-alone and letrozole plus FSH groups compared with tamoxifen plus FSH ($P<.05$). The mean follow-up for all study subjects was 554 ± 31 days, and there was no difference in risk of breast cancer recurrence between the control group and women who underwent IVF (23).

Building on our previous findings, we sought to compare the efficacy of the AI plus gonadotropin protocol in breast cancer patients and a standard IVF protocol in noncancer patients. Forty-seven women with stages I–IIIA breast cancer desiring fertility preservation underwent COH with the use of letrozole and FSH. Letrozole (5 mg) was started on cycle day 2 or 3. Daily injections of FSH (150–300 IU/d) were added 2 days later. All medications were discontinued on the day of hCG administration. Letrozole was reinitiated after oocyte retrieval and continued until E_2 levels fell to <50 pg/mL. Fertilization was achieved by IVF–intracytoplasmic sperm injection (ICSI). An age-matched control group of 56 women undergoing IVF for tubal disease was retrospectively identified. Briefly, the stimulation protocol for the control group consisted of a GnRH agonist during the preceding luteal phase with the addition of gonadotropins on cycle day 2 or 3. Total oocytes, mature oocytes, fertilization rate, number of embryos, and length of stimulation were similar between the two groups. Peak E_2 levels were significantly lower in the letrozole plus FSH group compared with the control group (483 ± 278.9 pg/mL vs. 1,464.6 ± 644.9 pg/mL; $P<.001$). In addition, there was a 44% reduction in the total gonadotropin requirement in the letrozole plus FSH group compared with the control group. Given these data, we concluded that letrozole plus FSH for COH offered breast cancer patients undergoing fertility preservation yields similar to standard protocols while minimizing the risk of high estrogen exposure and reducing the amount of gonadotropins required (24).

To further characterize the risk of COH using letrozole and FSH on the risk of breast cancer recurrence, we enrolled 215 breast cancer patients into a prospective nonrandomized controlled study from January 2002 to April 2007. Seventy-nine women elected to undergo COH with letrozole and FSH, and the remaining 136 women declined fertility preservation and served as control subjects. Letrozole (5 mg) was start on cycle day 2 or 3. Daily injections of FSH (150–300 IU/d)

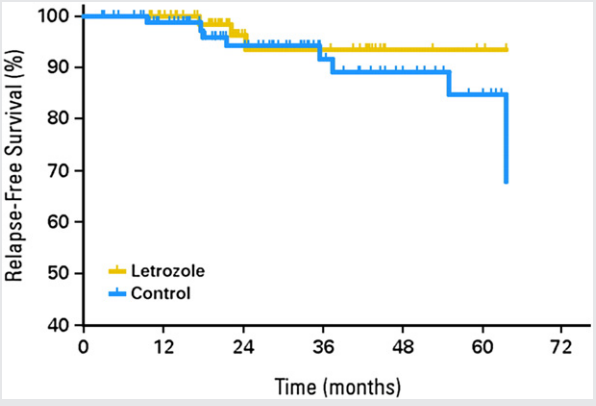
were added 2 days later until hCG administration. Letrozole was restarted on the day of oocyte retrieval to prevent a rebound increase in E_2 levels. The mean follow-up after chemotherapy was 23.4 months (range 7.5–63.3 months) in the letrozole plus FSH group and 33.05 months (range 4.5–63.3 months) in the control group. There was no difference in relapse-free survival between the two groups (hazard ratio 0.56, 95% CI 0.17–1.9; Fig. 1). Based on these findings we concluded that the use of letrozole plus FSH for COH for fertility preservation in breast cancer doesn't appear to significantly raise the risk of breast cancer recurrence in the short term. We also cautioned that longer follow-up was needed before definitive judgment can be rendered on the risk of breast cancer recurrence attributed to COH with the use of AIs and gonadatropins (25).

Traditionally, breast cancer survivors only have time to undergo one cycle of COH before initiating adjuvant chemotherapy, which typically occurs after breast surgery (13). In the event of a poor response, multiple cycles are often not feasible owing to time constraints. We sought to determine if early referral for fertility preservation could improve cycle outcomes. We secondarily analyzed data from a prospectively collected database investigating the risk of COH in women with breast cancer. Ninety-three women were included in the study and divided into two groups based on timing of the referral. Thirty-five women were referred before breast surgery and 58 after surgery. Women referred before breast surgery were significantly more likely to undergo a second cycle of COH than women referred after surgery ($P<.001$). This resulted in significantly higher numbers of oocytes retrieved and embryos cryopreserved in the presurgical group compared with the postsurgical group ($P<.001$). Interestingly, the time from initial diagnosis of breast cancer to the initiation of chemotherapy was significantly shorter in the presurgical group than in the postsurgical group (83.9 ± 24.3 days vs. 107.8 ± 42.9 days; $P<.045$). These data suggest that early referral before breast surgery allows breast cancer survivors to undergo fertility preservation with potentially multiple cycles without delaying the initiation of adjuvant chemotherapy (8). Madrigrano et al. (14) reported similar findings from a retrospective chart review of 23 women referred for fertility preservation before breast cancer treatment. On average, women completed ovarian stimulation and oocyte retrieval within a 2-week period (mean 11.5 days, range 9–20 days), further confirming that fertility preservation can be successfully integrated into a multidisciplinary breast cancer treatment model (14).

Although the importance of early referral for fertility preservation can not be overstated, fertility specialists are occasionally confronted with insufficient time for tradition COH. Recent evidence suggests that oocytes can be successfully retrieved in cancer patients within 2 weeks regardless of the menstrual cycle day at the time of initial presentation (35, 36). Von Wolff et al. described a novel protocol for cancer patients that initiated ovarian stimulation during the luteal phase of the menstrual cycle. Compared with cancer patients stimulated during the follicular phase, the luteal-phase group had a similar number of aspirated oocytes, number of viable metaphase II oocytes, and fertilization rate (36). Likewise, we developed a stimulation protocol for women with breast cancer who presented for emergency fertility preservation. Owing to their late referral, there was insufficient time to wait for the onset of their next menstrual cycle. Our random-start COH protocol included AIs, FSH, and GnRH antagonists. Stimulation commenced on menstrual cycle day 11, 14, or 17, respectively, in the three patients included in our case series. Because a premature LH surge can occur at very low E_2 levels even during an AI cycle, we recommend starting a GnRH antagonist when one leading follicle measures >13 mm. Seven to ten embryos were frozen with favorable fertilization rate in the three cases. Although the data are extremely limited, our findings are encouraging for breast cancer patients presenting for emergency fertility preservation (37, 38). Additional studies are needed to determine if oocytes and embryos obtained from late follicular or luteal phase ovarian stimulation cycles have pregnancy rates similar to those originating from conventional IVF cycles.

Maximizing the number of embryos and oocytes cryopreserved during a fertility preservation cycle is extremely important, not only because of time constraints but also to increase the chance of future pregnancies. One strategy to increase the embryo and oocyte yield per cycle has been to use higher doses of gonadotropins. We evaluated the efficacy of ovarian stimulation with the use of higher starting doses of gonadotropins. This was a secondary analysis of previously collected data. We specifically compared ovarian response to a low-dose protocol (150 IU FSH) versus a higher-dose protocol (>150 UI). One hundred fifty-one patients met the inclusion criteria, of which 34 were in the low-dose group and 117 in the higher-dose group. Although the number of follicles >17 mm was greater in the higher-dose group, there was no difference in number of oocytes (13.3 ± 8.7 vs. 12.3 ± 8.0) or embryos (6.3 ± 4.7 vs. 5.4 ± 3.8) generated between the two groups. Notwithstanding the small sample size, our data suggests that initial higher doses of FSH do not significantly improve cycle outcomes in women undergoing fertility

FIGURE 1



Relapse-free survival in women with breast cancer stimulated with letrozole versus control group. Kaplan-Meier plot (hazard ratio 0.56, 95% CI 0.17–1.9). Reproduced with permission from Azim AA et al., J Clin Oncol 2008;26:2630–5 (23).

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preservation with AIs (39). Furthermore, a recent meta-analysis reported that the optimal daily FSH dose was 150 IU in presumed normal responders <39 years old undergoing IVF, because this dose was associated with similar pregnancy and embryo cryopreservation rates compared with higher doses (40). In addition, our finding appears to be consistent with the theory that higher doses of FSH may stimulate the recruitment of chromosomally abnormal or incompetent oocytes (41). Nevertheless, depending on the age, body mass index, and antral follicle counts, doses of >150 IU may be required. One strategy we use to tailor the gonadotropin dose is to measure serum FSH levels during stimulation. In preliminary studies we found that the optimal serum FSH levels run from 21 IU/L to 30 IU/L (42).

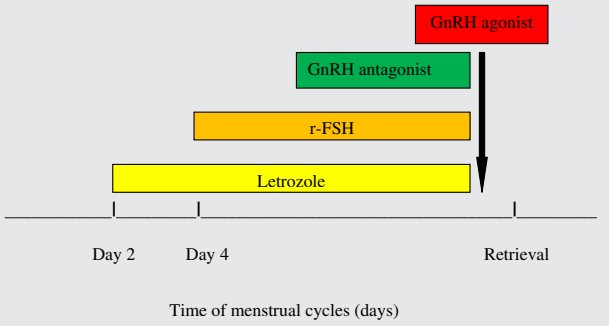
hCG trigger carries the well known risk of inducing ovarian hyperstimulation syndrome (OHSS) (43, 44). In addition, given its longer half-life compared with endogenous LH, hCG potentiates the endogenous production of estrogen during the luteal phase, which is not desirable in breast cancer patients (45, 46). Furthermore, development of OHSS may be even more serious in cancer patients because of their underlying risk of thromboembolism and the potential need to delay chemotherapy until resolution of the OHSS. We have also encountered practical difficulties with oncologists accidently delaying chemotherapy owing to a false positive pregnancy test induced by the hCG trigger (47). Therefore, in recent years we have preferred GnRH agonist (GnRHa) trigger instead of hcG trigger in cancer patients.

In a recent study we compared GnRHa and hCG as the trigger for final oocyte maturation in fertility preservation cycles. All women were started on 5 mg letrozole daily on cycle day 2 or 3 and FSH (150–300 IU/d) added 2 days later. When at least two leading follicles reached 20 mm, oocyte maturation was triggered with either 1 mg leuprolide acetate (Ferring Pharmaceuticals) or 5,000–10,000 IU hCG (Organon) or 250 μg recombinant hCG (Serono). Forty-seven women were triggered with hCG and 27 leuprolide acetate. The leuprolide acetate trigger resulted in a higher number of mature oocytes and cryopreserved embryos compared with the hCG group. Although the peak E_2 levels on the day of trigger were significantly higher in the leuprolide acetate group than in the hCG group (695.5 $\pm$ 539 pg/ml versus 472.6 $\pm$ 345.5 pg/ml; $P=.044$), there was a significantly faster drop in E_2 levels in the leuprolide acetate group than in the hCG group (89.5 $\pm$ 6.3% vs. 79.0 $\pm$ 13.4%; $P=.013$). In addition, there was a significantly lower rate of moderate/severe OHSS in the leuprolide acetate group compared with the hCG group (3.7% vs. 21.3%; $P=.047$) (48). We concluded that given the improved cycle outcomes while reducing the overall exposure to elevated estrogens and the risk of OHSS, GnRHa may be considered as the first line agent for triggering final oocyte maturation (47). Other advantages of using GnRHa as a trigger in cancer patients is that if they have time to undergo consecutive cycles, their luteal phase can be shortened, it can be used in a random-start protocol during the luteal phase, and the risk of residual cysts may be lower (49). Therefore, in our current practice we primarily use GnRHa instead of hCG trigger in fertility preservation cycles for women with breast cancer. The trigger, however, must be confirmed the next morning by measuring serum FSH, LH, and P levels. Depending on how long after the trigger the blood work is completed, LH and/or FSH levels may be found to be low but an elevation in P to postovulatory levels will confirm that the trigger has occurred. The tests should be processed as soon as possible so that an hCG trigger can be given should there be a lupron trigger failure.

Some women with breast cancer may be at a disadvantage when it comes to ovarian stimulation. Domingo et al. (50) studied the ovarian response of cancer patients to COH before chemotherapy and compared it with a historical cohort. A total of 223 women with cancer were included in their study and divided into two groups, hormonally dependent cancer and nonhormonally dependent cancer. The historical group consisted of healthy age-matched control subjects presenting for IVF because of severe male-factor infertility. The hormonally dependent group, of which breast cancer was the most prominent type, was stimulated with 5 mg letrozole daily starting on menstrual cycle day 2 or 3. After 2 days, daily injections of FSH (150–220 IU/d) (Gonal F; Serono) were added. A GnRH antagonist was administered when the leading follicle measured 14 mm. Final oocyte maturation was triggered using a GnRHa. A poor response, defined as four or fewer oocytes, was more frequently encountered in the cancer group than in the control group (21.2% vs. 2.6%; $P<.001$). When the hormonally dependent group was compared with the nonhormonally dependent group, the hormonally dependent group was significantly more likely to have a poor response to COH (odds ratio 2.99, 95% CI 1.49–6.02). The authors concluded that patients with hormonally dependent cancer are more likely to have a poor response to COH than patients with nonhormonally dependent cancer (50). A limitation of that study is that letrozole was used only to stimulate the hormonally dependent group; therefore, the poor response may be secondary to a difference in the stimulation protocol (50). In fact, we reported that improved oocyte maturation and fertilization rates can be achieved in letrozole cycles when ovulation is triggered at 19–20 mm rather than the traditional 17–18 mm (24). This difference seems to be the result of letrozole mediated follicular fluid dynamics (24). Supporting this clinical observation is that mice follicles exposed to aromatase inhibitors reach the antral stage earlier (51).

Strategies to identify poor responders before stimulation have the potential to make significant contributions to the field of fertility preservation for breast cancer survivors. *BRCA* genes play an essential role in double-stand DNA break repair; therefore, *BRCA* germline mutations are associated with an increased risk of breast and ovarian cancers (52). Given that DNA repair is deficient in patients with *BRCA* mutations, we hypothesized that these patient's oocytes may be more prone to DNA damage, clinically manifesting as diminished ovarian reserve. To test our hypothesis, we compared ovarian response to letrozole plus FSH in breast cancer survivors with and without *BRCA* germline mutations. This was a secondary analysis of a prospective cohort study of women with breast cancer undergoing fertility preservation. A low ovarian response was defined as the retrieval of four or fewer oocytes in women <38 years old. *BRCA* mutation–positive women were significantly more likely to respond poorly to ovarian stimulation than *BRCA* mutation–negative women

FIGURE 2



Aromatase inhibitor protocol for fertility preservation cycles for women with breast cancer.

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(33.3% vs. 3.3%; $P=.014$). A subgroup analysis revealed *BRCA1* mutation carriers were 38 times more likely to have to a low response compared with *BRCA* mutation–negative women (95% CI 4.1–353.4; $P=.001$). This study was the first to suggest a possible clinical association between *BRCA* germline mutations, deficient DNA repair, breast/ovarian cancer risk, and diminished ovarian reserve (53). Our findings may also explain the higher rates of poor response to COH in women with hormonally dependent cancer encountered by Domingo et al. (50). Larger studies are warranted to further explore the clinical impact of *BRCA* germline mutations on fertility in the general population.

Anastrozole is another third-generation AI that is used in the treatment of breast cancer. In 2007, we reported our findings of a prospective sequential cohort study investigating the potency of anastrozole (Arimidex; AstraZeneca) compared with letrozole (Femara; Novartis) to suppress E_2 levels in breast cancer patients undergoing COH. Women received either 5 mg letrozole or 2–10 mg anastrozole daily. The anastrozole dose was started at 2 mg and increased 1–2 mg per day to suppress E_2 levels. The maximum daily dose of anatrozole was 10 mg. The study was prematurely terminated when interim analysis revealed that anastrozole failed to adequately suppress E_2 levels despite gradually increasing the dose of anastrozole to a maximum of 10 mg daily. Of note, there was no significant difference in length of stimulation, number of oocytes retrieved, fertilization rate, and number of embryos cryopreserved. Given the need to specifically minimize the risk of elevated E_2 in breast cancer survivors undergoing COH, we recommended against the routine use of anastrozole in fertility preservation cycles (54). The lack of efficacy of anastrozole in suppressing E_2 in the short term is probably due to its slightly lower efficacy in suppressing aromatase as well as its slower onset of action (55, 56). In addition,

TABLE 1

Summary of included studies.

Study ID	Study design	Population (n)	Main outcome(s)	Findings
Oktay et al., 2005	Prospective cohort	7 tamoxifen + FSH 11 letrozole + FSH 12 tamoxifen	Mature oocytes Embryos Peak E_2 levels	Tamoxifen + FSH and letrozole + FSH had significantly greater number of mature oocytes and embryos compared with tamoxifen alone. Letrozole + FSH had the lowest peak E_2 levels.
Oktay et al., 2006	Retrospective, age-matched cohort	47 letrozole + FSH 56 GnRHa + gonadotropins (control group)	Mature oocytes Embryos	No difference in mature oocytes or embryos. Peak E_2 levels were significantly lower in the letrozole + FSH group.
Azim et al., 2008	Prospective cohort	79 letrozole + FSH 136 declined IVF (control group)	Risk of cancer recurrence	No difference in relapse-free survival.
Lee et al., 2010	Prospective cohort	35 fertility preservation (FP) before surgery 58 FP after surgery	Mature oocytes Embryos Number of cycles	Women referred before surgery had significantly more oocytes and embryos and had 2 cycles of FP.
Lee et al., 2012	Prospective cohort	34 Low-dose FSH + letrozole 117 higher-dose FSH + letrozole	Mature oocytes Embryos	No difference.
Oktay et al., 2010	Retrospective cohort	27 GnRHa trigger 47 hCG trigger	Mature oocytes Embryos OHSS rate	GnRHa trigger had significantly greater number of mature oocytes and embryos while reducing the risk of OHSS.
Domingo et al., 2012	Retrospective, age-matched cohort	66 nonhormonally dependent cancer 142 hormonally dependent cancer 97 standard IVF (control group)	Retrieved oocytes	Hormonally dependent group had a significantly poorer response to stimulation.
Oktay et al., 2009	Prospective cohort	14 *BRCA* mutation positive 33 *BRCA* mutation negative	Retrieved oocytes	BRCA mutation–positive women were significantly more likely to have fewer retrieved oocytes.
Oktay et al., 2010	Prospective cohort	32 letrozole + FSH	Maturation of immature oocytes	Mature oocyte yield was increased by 45% using in vitro maturation.

Note: GnRHa = GnRH agonist; OHSS = ovarian hyperstimulation syndrome.

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some have raised concerns regarding the potential teratogenic effects of AIs on early fetal development. A large study of more than 900 newborns conceived on either clomiphene citrate or letrozole reported no increased risk of congenital malformations in newborns conceived on AIs (57). Furthermore, the half-life of letrozole is ~30–60 hours and therefore should be effectively cleared from the body at the time of implantation (58).

In vitro maturation (IVM) of immature oocytes has been another exciting development recently. Advantages of IVM over conventional stimulation include increased flexibility, avoidance of large doses of gonadotropins, decreased costs associated with medications, and reduced exposure to elevated levels of estrogen (59). A small percentage of oocytes retrieved during routine IVF are immature and typically discarded. We sought to explore the utility of IVM to improve oocyte or embryo yield for breast cancer patients undergoing fertility preservation (60). That study also was a secondary analysis of a prospectively collected database of women with breast cancer undergoing fertility preservation with the use of AIs. Following our standard ovarian stimulation protocol as described above, the cumulus was partially removed after oocyte retrieval. Immature oocytes at germinal vesical (GV) stage were placed in the IVM medium for 24 hours. The IVM medium was based on the sequential IVF medium (G2; Vitrolife) supplemented with 75 mIU/mL FSH (Organon), 10 mg/mL epidermal growth factor (Sigma-Aldrich), and 0.5 mg/L insulin-transferrin-selenium (Sigma-Aldrich). ICSI was performed on all of the oocytes, and all of the embryos were frozen at the pronuclear phase. Thirty-two patients were included in the study, and each patient only contributed one cycle to study. No cycle cancellations occurred. Of the 464 oocytes retrieved, 274 were mature, 174 were in the GV or metaphase I stage, and 16 were degenerate. Of the 174 immature oocytes placed in the IVM medium, 125 matured, increasing the mature oocyte yield by 45% (61). These initial data suggest that IVM can be a useful tool to further increase the future reproductive potential of breast cancer survivors (60, 61). Because patients in this cohort will likely be on tamoxifen for 5 years following chemotherapy, it may take years before we can report on their pregnancy outcomes.

In conclusion, preliminary studies have demonstrated that a letrozole plus gonadotropin protocol, summarized in Figure 2, is effective for safely inducing COH in women with breast cancer before initiating adjuvant chemotherapy. However, COH should always be initiated in conjunction with the patient's oncologist. A list of all reviewed studies is summarized in Table 1. Future areas of research should include identifying the mechanism of poor response in *BRCA* mutation carriers, developing strategies for improved outcomes with the use of IVM, and pregnancy outcomes. Further studies are needed also to study the effectiveness of this protocol for estrogen-sensitive conditions other than breast cancer (62). There have been exciting new developments in the field of fertility preservation in recent years, and to reflect this change American Society of Clinical Oncology is in the process of revising its guidelines on fertility preservation previously published in 2006 (6).

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