High Yield of Oocytes without an Increase in Circulating Estradiol Levels in Breast Cancer Patients Treated with Follicle-Stimulating Hormone and Aromatase Inhibitor in Standard Gonadotropin-Releasing Hormone Analogue Protocols

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ABSTRACT: **Background:** Adjuvant/neoadjuvant chemotherapy in breast cancer patients may be associated with amenorrhea and a marked reduction in ovarian reserve.

Objectives: To assess the use of letrozole with follicle-stimulating hormone (FSH) in gonadotropin-releasing hormone (GnRH) analogue protocols, based on reported attempts to avoid the estradiol (E2) increase during controlled ovarian hyperstimulation for embryo cryopreservation in breast cancer patients using a combination of low dose FSH and aromatase inhibitor (letrozole) in a GnRH-antagonist protocol.

Methods: Twenty-four breast cancer patients were treated with recombinant FSH (150–450 U/day) and letrozole (5 mg/day) in a long GnRH-agonist (n=7) or GnRH-antagonist (n=17) protocol. After oocyte retrieval, insemination and/or intracytoplasmic sperm injection was performed. The embryos were frozen.

Results: The average interval from surgery to oocyte retrieval was 40 days. Average duration of treatment was 9.6 days and mean peak E2 level 1342 ± 1091 pmol/L, yielding 16.0 ± 16.3 oocytes (range 0–82). Mean fertilization rate was 69.5 ± 20.4% and mean number of embryos cryopreserved 10.3 ± 9.3. More oocytes were retrieved with the long GnRH protocol, but the difference was not statistically significant (24.8 ± 24.6 vs. 12.0 ± 8.8 pmol/L, $P = 0.07$).

Conclusions: As previously reported, ovarian stimulation with letrozole and FSH, in both the long GnRH-agonist and GnRH-antagonist protocols, is apparently effective in breast cancer patients and spares them exposure to high E2 levels.

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KEY WORDS: breast cancer, in vitro fertilization (IVF), letrozole, fertility preservation

Breast cancer is the most common cancer in women in developed countries. Approximately 2% of cases occur in women aged 20–34 years and 11% in women aged 35–44 years [1]. With the significant improvement in the survival of breast cancer patients in recent years, the potential late effects of treatment and the impact on quality of life have become increasingly important. Adjuvant/neoadjuvant chemotherapy may be associated with amenorrhea and a marked reduction in ovarian reserve, depending on the patient's age and the class and dose of drugs used [2-4]. Moreover, in patients with estrogen-positive disease, the need to delay pregnancy for several years during hormone treatment (tamoxifen with or without gonadotropin-releasing hormone agonists) places an additional burden on the already diminished ovarian reserve.

To avoid the potential risks of rising estradiol levels during controlled ovarian hyperstimulation in women with breast cancer, Oktay et al. [5-7] described an ovarian stimulation protocol where the aromatase inhibitor letrozole was administered before in vitro fertilization for embryo or oocyte cryopreservation. They found that E2 remained at levels similar to those in unstimulated cycles, and the oocyte and embryo yields were comparable to those of standard ovarian stimulation protocols. Since these patients usually undergo only a single IVF attempt before commencing chemotherapy, it is crucial that as many cryopreserved embryos as possible be obtained in this cycle for future use.

In their pioneer study, Oktay and co-authors [6] used a low dose (150 U/day) of recombinant follicle-stimulating hormone in a GnRH-antagonist protocol. The aim of the present study was to evaluate the combination of letrozole with higher doses of FSH in long GnRH-agonist and GnRH-antagonist protocols.

E2 = estradiol
IVF = in vitro fertilization
GnRH = gonadotropin-releasing hormone

PATIENTS AND METHODS

The study population consisted of patients with breast cancer who between 2005 and 2009 were referred for consultation for fertility preservation before adjuvant chemotherapy. All data were collected prospectively by one physician (A.B-H.). The present retrospective analysis was approved by the local institutional review board. Only women with stage III cancer or lower were treated.

Ovarian stimulation was performed with recombinant FSH (Gonal F, Serono, Geneva, Switzerland) at a starting dose of 150–375 IU/day in a long mid-luteal GnRH-agonist or GnRH-antagonist protocol. The choice of protocol was made on an individual basis by the treating physician (A.B-H.) according to the expected time of menstruation. The long GnRH protocol consisted of daily injections of Decapeptyl® (Ferring, GmbH, Kiel, Germany) 0.1 mg or a depot injection of Decapeptyl 3.75 mg at the mid-luteal phase. Down-regulation was confirmed after menstruation and was followed by gonadotropin stimulation. The GnRH-antagonist protocol consisted of daily gonadotropin stimulation from day 3 or 4 of menstruation followed by daily injections of Cetrotide® 0.25 mg (Serono) when the leading follicle reached 14 mm and continued until the day of human chorionic gonadotropin injection. Letrozole (Femara®, Novartis Pharma Sein AG, Basel, Switzerland) was started at 5 mg/day on the second day of the menstrual cycle and continued until the day of hCG trigger. The starting gonadotropin dose was determined on the basis of the patient's age and body mass index according to our standard departmental protocols. A baseline pelvic ultrasound assessment was performed on cycle day 2, and ultrasound and E2 monitoring were then performed every 2 to 4 days after the initiation of gonadotropins. Intramuscular hCG was administered when at least two follicles reached at least 18–20 mm in diameter. Transvaginal oocyte retrieval was performed approximately 36 hours after hCG administration. Oocytes were fertilized by intracytoplasmic sperm injection or standard IVF insemination, depending on the semen parameters. Embryos were cryopreserved by slow freezing at the two-pronucleus stage. Letrozole was reinitiated on the day of oocyte retrieval to prevent a rebound increase in E2 levels and continued for 2–4 days thereafter.

Data were managed and analyzed with the SPSS statistical package, version 15 for Windows. Mann-Whitney and chi-square tests were used, as appropriate. A *P* value less than 0.05 was considered significant.

Table 1. IVF cycle characteristics in breast cancer patients in the long GnRH agonist and GnRH-antagonist protocols

	Long GnRH-agonist protocol (n=7)	GnRH-antagonist protocol (n=17)	*P* value
Age (yrs) (range)	31.8 ± 3.3 (28–39)	32.2 ± 4.6 (24–41)	0.84
Total FSH dose (IU)	2971 ± 1125	2541 ± 1019	0.34
Duration of stimulation (days)	10.5 ± 2.6	9.2 ± 1.8	0.20
FSH dose/day (IU) (range)	275 ± 45 (225–337)	269 ± 80 (150–450)	0.84
Peak estradiol (pmol/L) (range)	1466 ± 1047 (80–3187)	1248 ± 1138 (244–5000)	0.70
No. of retrieved oocytes (range)	24.8 ± 24.6 (0–82)	12.0 ± 8.8 (3–31)	0.07
No. of retrieved oocytes* (range)	± 9.6 (0–30)	12.0 ± 8.8 (3–31)	0.28
Fertilization rate (%)			
IVF	60.6 ± 27.9	58.6 ± 33.5	0.88
ICSI	73.0 ± 15.2	74.7 ± 27.1	0.89
Total	74.7 ± 27.1	73.0 ± 22.0	0.18
No. of frozen embryos	14.2 ± 13.3 (0–45)	8.2 ± 6.4 (2–27)	0.66
No. of frozen embryos*	9.8 ± 5.1 (0–16)	8.2 ± 6.4 (2–27)	0.54

*Excluding one patient who hyper-responded to gonadotropins with 82 retrieved oocytes
ICSI = intracytoplasmic sperm injection

RESULTS

Between 2005 and 2009, 24 patients with breast cancer (mean age 32.1 ± 4.1 years, range 24–41) underwent ovarian stimulation with gonadotropins and letrozole in a long GnRH-agonist protocol (n=7) or a GnRH-antagonist protocol (n=17) [Table 1]. The mean interval from the definitive surgery to oocyte retrieval was 40 days (range 22–49). Patients were treated for an average of 9.6 days, with peak E2 levels of 1342 ± 1091 pmol/L (range 80–5000), yielding a mean of 16.0 ± 16.3 oocytes/cycle (range 0–82). In one woman, no oocytes were retrieved. The overall rate of fertilization was 69.5 ± 20.4%; a mean of 10.3 ± 9.3 embryos (range 0–45) was cryopreserved. One woman on the long GnRH-agonist protocol hyper-responded to gonadotropins (225 IU/day of FSH injections) with 82 retrieved oocytes (peak E2 3187 pmol/L). Ovarian hyperstimulation syndrome was ruled out on clinical, sonographpic and laboratory follow-up. The number of retrieved oocytes was higher in women in the long GnRH protocol than the GnRH-antagonist protocol, but the difference was not statistically significant [Table 1]. During follow-up of 20–52 months, cancer recurrence rate was found in 2 of 24 patients.

DISCUSSION

Based on previous reports, the present study showed that ovarian stimulation with recombinant FSH and letrozole seems to be effective in patients with breast cancer in both the long GnRH agonist and GnRH-antagonist protocols, while preventing the surge in E2 levels. The major limitations in the

HCG = human chorionic gonadotropin

current study were the small sample size and the lack of a control group. However, since a real control group (without aromatase inhibitor) cannot be treated for obvious reasons and a larger number of young breast cancer patients who wish for fertility preservation is difficult to achieve, our study still adds important data to those previously reported by others.

Oktay et al. [6] were the first to describe the use of letrozole in the GnRH-antagonist protocol in a study of 29 patients with breast cancer. Their analysis included 33 ovarian stimulation cycles with tamoxifen 60 mg/day alone, tamoxifen 60 mg/day in combination with low dose FSH, or letrozole 5 mg in combination with low dose FSH (150 U/day). They found that the patients given letrozole and FSH had more follicles, more mature oocytes (8.5 ± 1.6), and more embryos (5.3 ± 0.8) than the other groups. As expected, peak E2 levels were lower with letrozole (1370 ± 205 pmol/L). In our study, patients in the GnRH-antagonist group were treated with higher doses of FSH (150–450 U/day, mean 270 U/day). This resulted in a higher number of retrieved oocytes (12.0 ± 8.8) and frozen embryos (8.2 ± 6.4) than the lower-dose schedule [6] but similarly low levels of peak E2 (1248 ± 1138 pmol/L). The mean total FSH dose was 2697 ± 1050 U, which is higher than reported by Azim et al. [8] (1469 ± 741 U). The long GnRH-agonist protocol was associated with a slightly higher yield of retrieved oocytes and frozen embryos; but the difference from the GnRH-antagonist protocol was not statistically significant [Table 1].

One major concern is the long-term safety of IVF in patients with breast cancer. In their first study, Oktay and team [6] followed their patients for a mean duration of 554 ± 31 days (range 153–1441 days). The cancer recurrence rate was similar in the IVF and control groups (3/29 vs. 3/31 patients, respectively; hazards ratio 1.5, 95% confidence interval 0.29–7.4). The risk was not affected by cancer stage. In a recent follow-up report [9], cancer recurrence was compared among 79 women who elected to undergo ovarian stimulation with letrozole and gonadotropins for embryo or oocyte cryopreservation and 136 patients in whom no fertility-preserving procedure was performed (controls). The median follow-up after chemotherapy was 23.4 months in the study group and 33.05 months in the control group. The hazards ratio for recurrence after IVF was 0.56 (95% CI 0.17–1.9), and the survival rate was similar in the two groups. Given the similar peak E2 levels in our patients to those reported in these studies, we expect the long-term results to be the same.

Since most women undergo only one IVF cycle prior to chemotherapy, a higher yield of oocytes and embryos is desirable for fertility preservation. However, lower FSH doses are usually used in first cycles because of the risk of ovarian hyper-response and OHSS. On the basis of the present findings and the reports in the literature, we recommend the use of letrozole and FSH in the GnRH-antagonist protocol, so that GnRH-agonist can be used instead of hCG for final maturation of oocytes before oocyte retrieval [10]. In this manner, higher doses of FSH can be administered while avoiding the risk of OHSS [11], achieving a higher number and percentage of mature oocytes and a higher number of cryopreserved embryos or oocytes compared with hCG [12]. Importantly, pituitary suppression with a GnRH antagonist may result in a plateau or decrease in estradiol levels [13], another possible advantage in breast cancer patients. Since estradiol levels cannot be used for monitoring the magnitude of ovarian stimulation, frequent ultrasound evaluation of follicle count and size should be performed to prevent exaggerated ovarian hyper-response and OHSS, particularly in younger lean patients with high pretreatment antral follicle count.

It should be emphasized that the current use of letrozole in Israel for this indication is "Off Label," since in 2005 the manufacturer, Novartis Pharmaceutical, issued a statement to physicians advising that the use of letrozole in premenopausal women – and specifically its use for ovulation induction – is contraindicated. This warning was released following the 2005 annual meeting of the American Society for Reproductive Medicine, where an abstract presentation examined a relatively small number of letrozole pregnancies compared with a large control group of spontaneous conceptions [14]. The presenter suggested that the use of letrozole for infertility treatment might be associated with a higher risk of congenital cardiac malformation in newborns. However, more recently, Tulandi et al. [15] evaluated the incidence and type of congenital malformation among 911 newborns of mothers who had conceived with letrozole compared with a control group of infertile women who had conceived with clomiphene citrate. Their study demonstrated no difference in the overall rates of malformations or chromosomal abnormalities among the newborns of mothers who had conceived after letrozole or after clomiphene citrate treatments. Congenital cardiac anomalies in their study were statistically significantly less frequent in the letrozole group than in the clomiphene citrate group. Based on their data, the concern that letrozole use for ovulation induction could be teratogenic is unfounded [16].

In summary, despite the limitation of the low number of patients in the current study, as previously reported by others FSH can be used in IVF cycles for fertility preservation in patients with breast cancer when the potent aromatase inhibitor letrozole is added. This combination yields a high number of oocytes with low peak estradiol levels in both the long GnRH-agonist and GnRH-antagonist protocol, while sparing patients' exposure to high E2 levels.

CI = confidence interval
OHSS = ovarian hyperstimulation syndrome

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