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Twin delivery after IVF-ET with variable dose letrozole-FSH protocol of lower estradiol in a patient previously treated for breast cancer: a case report

Chang-Jun Zhang[1], Ying Zhang[1], Guang-Zhu Hu[2], Honglu Diao[1]

[1] Reproductive Medical Center, Renmin Hospital, Shiyan, Hubei; [2] Department of Thoracic and Cardiomacrovascular Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan, Hubei (China)

Summary

Objective: To present a case of twin pregnancy obtained by *in vitro* fertilization and embryo transfer (IVF-ET) with variable dose letrozole-FSH protocol of lower peak estradiol level, after treatment of carcinoma of the breast. *Materials and Methods:* A 34-year-old patient diagnosed with mucinous breast carcinoma undergoing assisted fertilization treatment after breast cancer operation and treatment including controlled ovarian stimulation (COS), oocyte retrieval, IVF, and embryo culture and transfer. *Results:* Four oocytes were obtained in three COS procedures in the three IVF cycle. All oocytes were fertilized. In the third cycle, two fresh embryos were transferred, and two healthy girls were born at 37 gestational weeks. *Conclusion:* Variable dose letrozole-FSH protocol can maintain lower peak estradiol levels and reduce estrogen exposure after breast cancer operation and chemotherapy.

Key Words: Breast cancer; Variable dose letrozole-FSH protocol; IVF-ET; Twin pregnancy.

Introduction

Breast cancer is the most common invasive cancer seen in women of reproductive age, with more than one million cases occurring annually worldwide [1]. One of every 228 women develops breast cancer before age 40, and approximately 15% of all breast-cancer cases occur during the reproductive years [2]. The survival rate of young breast cancer patient has increased in the recent years and it has become important to promote life quality of those women with post-cancer.

The majority of women diagnosed with early-stage breast cancer have an excellent long-term prognosis, which was given a combination chemotherapy, which includes gonadotoxic agents such as cyclophosphamide. As a result, a significant proportion of cancer survivors suffer from premature ovarian failure and infertility [3]. As greater emphasis is placed on the quality of life of breast cancer survivors due to higher survival rates, fertility preservation has become a key component of cancer care.

Fertility preservation in breast cancer patients remains unclear, for example, whether subsequent pregnancy has negative effects on the prognosis. If ovaries survive, doctors are not willing to manage the patient undergoing treatment by ovarian stimulation; increased physiological levels of sex steroids that result from ovarian stimulation for IVF may stimulate growth of malignant cells in a patient with a hormonally sensitive tumor, such as carcinoma of the breast [4].

Aromatase, an enzyme of the cytochrome P-450 superfamily and the product of the *CYP19* gene, can catalyze the reaction that converts androgenic substances to estrogen in many tissues, including granulosa cells of ovarian follicles [5]. Letrozole is a potent and highly selective third-generation aromatase inhibitor that was developed in the early 1990s. It competitively inhibits the activity of the aromatase enzyme and has a half-life of approximately 48 hours [5]. Because of its potent long-lasting suppression in the plasma levels of estradiol (E2), letrozole has been claimed to be better reagent than tamoxifen in the treatment of advanced-stage postmenopausal breast cancer [6]. Clinical studies in which letrozole was typically administered at doses of 2.5 to five mg for five days have also shown its benefit in ovulation induction alone or in combination with FSH. These studies also showed that the peak of E2 levels were lower with letrozole alone or in combination with FSH, compared to stimulation with FSH or clomiphene alone. Moreover, E2 levels have been found to be even lower than those seen in natural cycle when patients were stimulated with letrozole [7].

To reduce the estrogen exposure during ovarian stimulation in women with breast cancer, the present authors modified frequently-used protocols that used letrozole as an ovarian stimulant with adjusting dosage. Ovarian stimulation protocol by combining letrozole with low-dose FSH which dose was adjusted can reduce the maximum degree on E2 concentration during controlled ovarian stimulation (COS).

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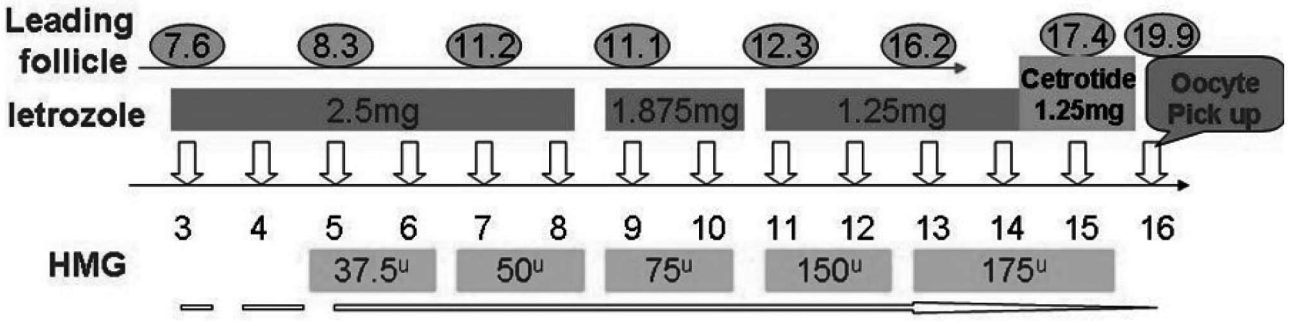

Figure 1. — The protocol of the third cycle with variable dose letrozole-FSH protocol in the third COS treated cycle. Letrozole was administered 2.5 mg/d from days 3 to 8, 1.875 mg/d from days 9 to 10, and 1.25 mg/d from days 11 to 14, respectively. HMG was administered 37.5 IU/d from days 5 to 6, 50 IU/d from days 7 to 8, 75I U/d from days 9 to 10, 150 IU/d from days 11 to 12, and 175 IU/d from day 13 to 15 respectively. The size of leading follicle was 7.6, 8.3, 11.2, 11.1, 12.3, 16.2, 17.4 and 19.9 mm on days 3, 5, 7, 9 11, 13, 15, and 16, respectively.

This case reports a patient who successfully achieved pregnancy and delivered twins by *in vitro* fertilization and embryo transfer (IVF-ET) using protocols above after treatment of carcinoma of the breast. This is the only such case reported to date.

Case Report

The patient 29-years of age, was found with a lump in her left breast by palpation in September 2006. Mammography was highly indicative of malignancy, and the carcinoma was confirmed by fine needle aspiration. A metastatic work-up was negative and the patient underwent immediate lumpectomy and axillary node dissection. Pathological examination revealed a 2.6-cm mucinous carcinoma without the microscopic focus of tumors in 19 nodes. The immunohistochemical test showed the tumor overexpressed ER and PR, Ki-67 negative, HER2, oncoprotein negative, lung-resistance related protein (LRP) negative, P-gp positive, and slightly positive GST-π. Serum E2 concentration and progesterone (P4) concentration were not detected. Chemotherapy with FAC (5-Fu, ADM, CTX) regimens were given ten days later after the operation; she underwent for periods of chemotherapy and 24 months of tamoxifen therapy sequentially. She was followed up both clinically as well as with liver and bone scans, and she had mammography performed every six months, and she remained disease-free with no evidence of recurrence of the tumor. Three years later after the operation, the patient attempted to become pregnant. A hysteroscopic tubal hydrotubation examination performed revealed a normal pelvic with patent fallopian tubes, and her serum D3, gonadotropins, E2, and P4 levels were normal. The results from ultrasound monitoring showed that she had no spontaneous ovulation, and her husband's semen analysis was normal. A provisional diagnosis of ovulation disorder infertility was therefore made by her gynecologist. The patient was adamant about having a baby and was referred to the present Reproductive Medical Center in June 2010. Despite being warned of the theoretical risk of provocation of disease, she was very keen to embark on IVF therapy.

Her first treatment cycle was begun at 34 years of age in July 2010. Letrozole (2.5 mg/day for menstrual days 3 to 5 and 3.75 mg/d from day 6 to 9) alone was administered to stimulate the ovaries. The largest follicle had a mean diameter of 18.1 mm on day 14 of her menstrual cycle. The levels of serum E2, P4, LH, FSH were 84 pg/ml, 1.47 ng/ml, 50.22 mIU/ml, and 17.66 mIU/ml, respectively, at 08:00 hours on day 14 of the menstrual cycle. Her urinary LH peak appeared timely correct on 21:00 hours, and the present authors considered that it was not necessary to trigger. One egg was retrieved at 08:00 hours on day 15 of her menstrual cycle, and after IVF, one embryo (eight-cell, grade 1) was frozen three days later considering that the embryo is not enough for ET.

The second IVF treatment cycle was in October 2010. Letrozole 2.5 mg/d alone was administered to stimulate the ovaries from menstrual days 3 to 9. The leading follicle had a mean diameter of 18.9 mm and there was another follicle with a diameter of 13.6 mm on day 13 of her menstrual cycle. The levels of serum E2, P4, LH, and FSH were 131 pg/ml, 1.89 ng/ml, 57.82 mIU/ml, and 17.48 mIU/ml at 08:00 hours, and 66 pg/ml, 9.47 ng/ml, 72 mIU/ml, and 23.14 mIU/ml, respectively, at 16:00 hours on day 13 of her menstrual cycle. Her nature LH surge appeared timely for the leading follicle. One oocyte was retrieved at 20:00 hours, and after IVF, one embryo (eight-cell, grade 2) was frozen three days later. The aforementioned two embryos were transferred to the patient's uterus in November 2010, but unfortunately, she did not achieve pregnancy.

In June 2011 the patient underwent the third IVF treatment cycle (detailed method is shown in Figure 1). She underwent a letrozole-FSH protocol ovarian stimulation. Letrozole was administered 2.5 mg/d from days 3 to 8, 1.875 mg/d from days 9 to 10, and 1.25 mg/d from days 11 to 14, respectively. Human menopausal gonadotropin (hMG) was administered 37.5 IU/d from days 5 to 6, 50I U/d from days 7 to 8, 75 IU/d from days 9 to 10, 150 IU/d from days 11 to 12, and 175 IU/d from days 13 to 15, respectively, according to the serum FSH level and the growth of follicles. Two follicles reach 17.4 and 14.9 mm in diameter by ultrasound examination on day 15 of her menstrual cycle. Cetrotide 1.25 mg was administered for the premature LH surge (LH 16.37 IU/ml) arising. The leading follicle had a mean diameter of 19.4 mm and another 16.5 mm on day 16 of her cycle. The level of serum E2, P4, FSH, and LH was 161 pg/ml, 3.42 ng/ml, 16.66 mIU/ml, and 35.45 mIU/ml, respectively, at 08:00 hours. The results were E2 95 pg/ml, P 2.92 ng/ml , FSH 13.04 mIU/ml, and LH 26.55 mIU/ml, respectively at 16:00 hours, which impressed the present authors that she had undergone a natural LH surge, without trigger. Two eggs were retrieved at 20:00 hours; after IVF two embryo (eight-cell grade 1, 4-cell grade 3) were transferred three days later. Two weeks later, the serum hCG titer was 1,175 IU/L, indicating a positive pregnancy test (pregnancy > 10I U/L).

An ultrasound scan (US) performed at six weeks after ET showed two intrauterine sacs with a separate positive fetal heart and the crown-lump length was consistent with the gestational age. A repeat US scans performed at 16 weeks showed two anatomically normal fetuses. The pregnancy continued uneventfully, and she delivered twins by cesarean section at 37 gestational weeks; two normal healthy girls weighing 2.85 kg and 2.75 kg, respectively. Mother and her two daughters both are currently healthy.

Discussion

Breast cancer accounts for one-third of all tumors seen in reproductive-aged women and affects tens of thousands of women each year in this age bracket. Definition of intrinsic subtypes has proved efficient in defining the prognosis for breast cancer patients [8]. Subtypes defined by clinico-pathological criteria are similar to but not identical to intrinsic subtypes and represent a convenient approximation. As summarized in an article published in 2011 [9], an approach used an immunohistochemical definition of ER and PR, the detection of overexpression, and/or amplification of the HER2, oncogene, and Ki-67 labeling index, a marker of cell proliferation, as the means of identifying tumor subtypes, although this method did not work out at that time. During the 12th St Gallen International Breast Cancer Conference (2011), the expert panel recommended that the clinicopathological markers described above were generally sufficient to guide therapeutic choices [9]. The panel strongly agreed that the 'luminal A' subtype, which the presented patient belonged to, was less responsive to chemotherapy, that chemotherapy was less useful in such patients, and essentially indicated endocrine therapy alone for patients with clinicopathologically classified 'luminal A' disease (except in defining high-risk cases), which expressed a preference for tamoxifen alone in premenopausal women. The patient can be considered fortunate to have recovered not only a timely and thorough lump excision in early stages of breast cancer, but also from lymph node metastasis that was found at the age of 34 years.

Decisions made regarding a pregnancy after breast cancer treatment among physicians is associated with considerable uncertainty. Meirow and Schiff postulated that patients who recover from ovulation failure after high-dose chemotherapy or radiotherapy treatments should try to conceive after a disease-free interval of a few years, but not less than six to 12 months after the treatment, due to the possible toxic effects of the therapy on growing oocytes [10]. However, completion of a pregnancy invokes two potentially opposing effects on the mother's subsequent risk of breast cancer, hence the dual effect of pregnancy. Delayed childbearing further increases this transient risk for subsequent breast cancer, with maternal age greater than 30 years at first birth resulting in both an elevation of the peak incidence in the initial year's post-partum and a long tail effect of increased risk persisting for 30-50 years post-partum [11, 12]. Müller *et al.* retrospectively compared 438 patients who became pregnant after a diagnosis of breast cancer with 2,775 control patients without pregnancies, and they found that women who had delivered at least ten months after the cancer diagnosis had a significantly lower mortality risk [13]. In terms of lifetime breast cancer risk, the age of 35 years acts as a critical breakpoint, prior to this age full-term pregnancy offers women some degree of protection, but after this age, full-term pregnancy is associated with a permanent increase in breast cancer risk [14].

Since no woman can be denied the right to become a mother, the literature reports that in patients with hormone-positive cancers, tamoxifen can be preceded up to five years, during which a pregnancy is contraindicated [15]. The optimal timing of a subsequent pregnancy after breast cancer is unclear and depends on the patient's prognosis, age, and personal situation. While a five-year wait would be associated with a greater certainty of long-term disease-free survival, it was felt that a delay of two to three years after the cancer treatment was appropriate, so that the period associated with the greatest risk of recurrence would be passed before a pregnancy [16].

Aggressive chemotherapy (AFC) has improved the life expectancy for reproductive-age women with breast cancer, but it often causes infertility or premature ovarian failure due to the destruction of the ovarian reserve. AFC appeared to be useful to counsel breast cancer patients before ovarian stimulation for fertility preservation [17]. The number of antral follicles of the present patient was eight, in range of normal value, which indicates normal ovarian response.

It has been clearly shown that an increase in E2 stimulates breast cancer cell growth during controlled ovarian hyperstimulation may not be safe even at low concentrations [18]. Thus, the prediction of stimulation results before COS is clinically meaningful and helpful. The use of aromatase inhibitors for ovulation induction was first reported in 2001; letrozole was reported to provide better results and with 50% lower E2 levels than clomiphene in ovulation [19]. Considering these factors, the present authors use the letrozole only and attempted to change the dose of letrozole during the first two cycles. Given that we obtained an egg in each cycle, in order to get more embryos for embryo transplantation, we decided to set up the third cycle with the modified frequently-used letrozole-FSH protocol. Indeed, the combined letrozole-FSH protocol resulted in level of peak estradiol close to that seen in unstimulated cycles, and breast cancer recurrence rates were not increased compared with controls [20]. The present authors modified frequently-used letrozole-FSH protocol in the third cycle, by combining letrozole with low-dose FSH in which both doses were adjusted according to hormones and dominant follicle size. Compared with the fixed dose letrozole protocol, it can lead to the minimum limited increase in E2 levels during COS. The present authors were very fortunate that the pregnancy had occurred and this is

first description of pregnancy using variable dose letrozole combining the FSH protocol in a gentle stimulated cycle.

A conducted study in the USA showed that cleavage state is not predictive of both implantation rate and live birth rate; embryo with less cell number is not suitable for ET [21]. However one study retrospectively reported that live birth rate was positively associated with increasing cell number up to eight cells (less six cells: 2.9%, six cells: 9.6%, seven cells: 15.5%, eight cells: 24.3%, and more than eight cells: 16.2%), but was negatively associated with maternal age, increasing fragmentation, and asymmetry scores [22]. In the present case the third cycle promoted a four-cell embryo for ET and reached a clinical pregnancy, showing that the four-cell embryo still has development potential.

Breast cancer survivors, such as the present case, who have successful pregnancies after treatment report that it helped normalize their life and the transition to wellness and having children improved the quality of their lives [23, 24]. However, the long-term risks of IVF treatment in patients who have had breast cancer remain unclear. At this time, the present report can prompt other physicians to consider the option of variable dose depending letrozole-FSH protocol in young women with breast cancer who want to become pregnant.

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Address reprint requests to:
HONGLU DIAO, Ph.D.
Reproductive Medical Center, Renmin Hospital
Hubei University of Medicine
No. 39 Chaoyang Middle Road
Shiyan, Hubei 442000 (China)
e-mail: hldiao1976@hotmail.com