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The effect of different cancer therapies on clinical outcomes of assisted reproductive technology in breast cancer patients

Mengmeng Zhang [a,f,1], Xueling Song [a,c,1], Caihong Ma [b], Xiumei Zhen [b], Hongbin Chi [b], Xiaowei Zhang [a,d], Rong Li [a,d,**], Jie Yan [a,d,*], Jie Qiao [a,e]

[a] Reproductive Medical Center, Peking University Third Hospital, Beijing, 100191, China
[b] Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, 100191, China
[c] Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, 100191, China
[d] Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproduction, Beijing, 100191, China
[e] Research Units of Comprehensive Diagnosis and Treatment of Oocyte Maturation Arrest, Chinese Academy of Medical Sciences, China
[f] Department of Obstetrics and Gynecology, Beijing Nuclear Industry Hospital, Beijing, 100045, China

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ABSTRACT

Objective: To evaluate the clinical outcomes of assisted reproductive technology (ART) on fertility preservation and infertility treatment in breast cancer patients who had undergone different cancer therapies.
Methods: 20 infertile females who had undergone breast cancer treatments during 2011–2018 were studied retrospectively. The patients were divided into two groups based on their cancer treatment methods and their needs of fertility preservation: the combined treatment group, who had both breast cancer surgery combining with any of the three treatments (adjuvant endocrine therapy, radiotherapy or chemotherapy), and the surgery only group. A group of infertile females without breast cancer history were used as a control group. An aromatase inhibitor Letrozole - based ovarian micro-stimulation protocol was used in females from the three groups for in vitro fertilization and embryo transfer. The ART clinical outcomes were evaluated by using the parameters of antral follicle count (AFC), the ratio of FSH/LH, oocyte retrieval number, 2 pronucleus(2 PN) fertilization rate, high-quality embryo rate, clinical pregnancy rate, and delivery outcome.
Results: The surgery only group had significantly lower ratio of FSH/LH than the combined treatment group and the control group. No significant difference on the ART clinical outcomes, evaluated by the aforementioned criteria, were found between the three groups.
Conclusions: Breast cancer surgery with adjuvant therapies, but not surgery alone, may damage ovarian function. The best time-limited window to preserve fertility for breast cancer patients is after surgery but before the initiation of adjuvant therapies. Importantly, the clinical outcomes of aromatase inhibitor-based ovary micro-stimulation in breast cancer patients are similar to that in non-breast cancer controls.

1. Introduction

The incidence of breast cancer has been increasing. It is the most common cancer affecting women worldwide and the leading cause of cancer-related deaths in women between the ages of 20 and 59 years. According to the American Cancer Society, the new breast cancer cases expected to be diagnosed in 2020 account for 30% of female malignancies [1]. About 12% of breast cancer patients are younger than 40

* Corresponding author. No. 49 Huayuan North Road, Hai Dian District, Beijing 100191, China.
** Corresponding author. No. 49 Huayuan North Road, Hai Dian District, Beijing 100191, China.
E-mail addresses: roseli001@sina.com (R. Li), yanjiebjmu@bjmu.edu.cn (J. Yan).

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[1] Mengmeng Zhang and Xueling Song contributed equally to this work.

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years old [2]. As young age is an independent risk factor for adverse outcomes, adjuvant chemotherapy is frequently recommended [3]. Among these patients, the proportion of women who have not given birth is increasing. At present, with the development of early diagnosis and improved multimodal therapy, breast cancer patients have the opportunity to meet their reproductive needs. But their fertility may be impaired by chemotherapy, which damages ovarian reserves. Population-based research shows that the pregnancy rate of breast cancer patients is only 3%, which is 40% lower than that of the general population [4]. With the increase in breast cancer incidence rate and the trend of fertility postponement, more breast cancer patients are seeking reproductive health care. ART is often used to prevent and treat infertility caused by chemotherapy [5].

Currently, breast cancer patients are divided into two groups according to the purpose of receiving ART intervention. One group needs preventive fertility preservation, which is mainly to reserve oocytes or embryos in advance for the fertility needs of patients after cure of cancer [5], while the other group needs treatment for infertility after breast cancer treatment [6]. The impact of different breast cancer treatments on ovarian function and pregnancy outcomes is still controversial. Both groups of patients need to use drugs for ovarian stimulation to obtain mature oocytes for cryopreservation or fertilization in vitro. However, the increase in serum estradiol levels caused by ovarian stimulation may accelerate the growth of breast cancer, which is a hormone-related disease [7]. To avoid the increase of estradiol, aromatase inhibitors have been used to stimulate ovary because aromatase inhibitor inhibits the increase of estradiol and do not increase the risk of breast cancer recurrence [8]. However, the impact of different breast cancer treatments on the efficacy and the safety of ovulation induction by using aromatase inhibitor is inconclusive, and the optimal timing of implementation of the micro-stimulation program and its clinical outcome of pregnancy are still controversial.

The purpose of this study was to retrospectively analyze the clinical outcomes of patients who underwent ART treatments of infertility, which had happened after their breast cancer therapies, to evaluate the effects of different cancer therapies on ART outcomes, and to explore the best timing for ART intervention in respect with the time frame of their breast cancer treatments.

2. Materials and methods

2.1. Participants

This study was reviewed and approved by the Institutional Animal Care and Use Committee of Peking University Third Hospital (Research License No. IRB00001052-16-31). 20 cases of infertility women after breast cancer treatment have received ART treatment in the reproductive center of the Peking University Third Hospital from December 2011 to

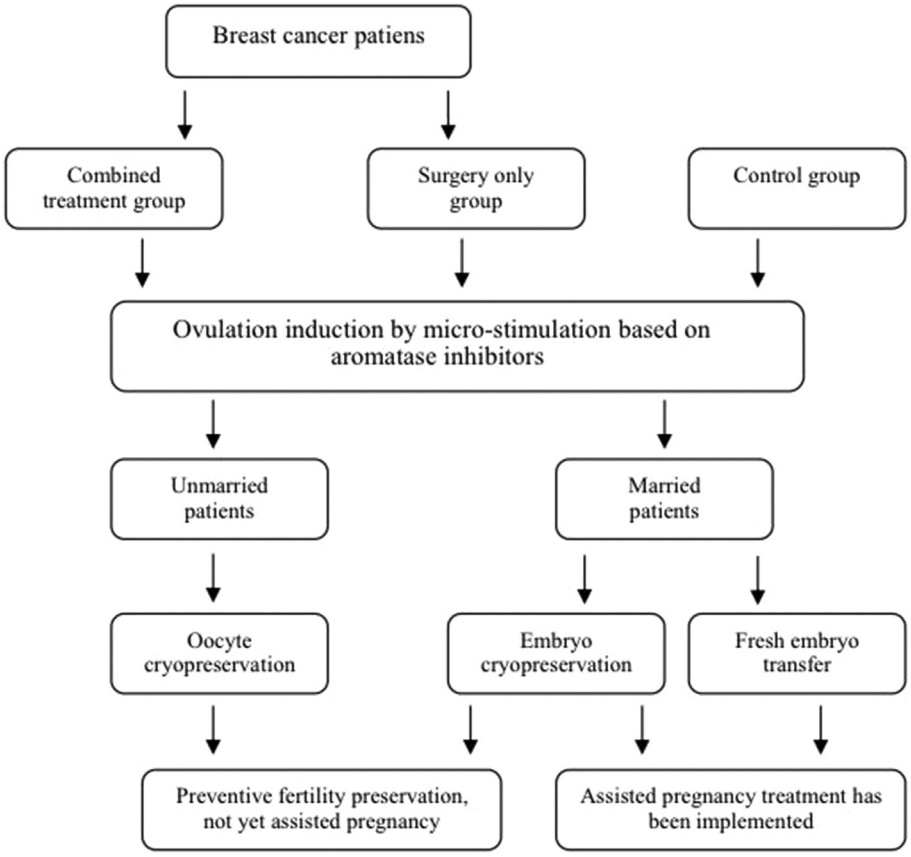

Fig. 1. Flowchart of patients grouping.

December 2018. All the patients were consent to participate in this study. None of these patients had children at the time of their diagnosis of breast cancer. The time when they came to the Center for ART treatment was 1 week to 8 years after the diagnosis of breast cancer. All had undergone breast conserving surgery or mastectomy, among which some of them had completed post-surgery adjuvant therapies, which included endocrine therapy in combination with radiotherapy or chemotherapy. According to the different treatment methods for breast cancer, the 20 patients were divided into two groups: the combined treatment group, which included nine patients who had received breast cancer surgery and adjuvant therapies (among of the 9 patients, three patients received breast conserving surgery and postoperative radiotherapy combining with endocrine therapy for five years, two patients of the received breast conserving surgery combined with radio-chemotherapy, and four patients underwent total mastectomy, 3 patients received chemotherapy and one received endocrine therapy), and the surgery only group, which included 11 patients who had undergone breast cancer surgery but without adjuvant therapy. 14 age-matched non-breast cancer infertile females who had had thyroidectomy were included as the control group. All females from the three groups had an aromatase inhibitor-based micro-stimulation for ovarian stimulation, which was followed by oocyte cryopreservation, embryo cryopreservation, or fresh embryo transfer based on the patients marital status (Fig. 1).

2.2. Superovulation and embryo transfer protocol

Ovarian stimulation on all patients from the three groups was performed using a micro-stimulation protocol based on aromatase inhibitor Letrozole in combination with gonadotropins. 2.5 or 5 mg/d Letrozole (Fu Rui, Jiangsu Hengrui) was administered orally starting on the second day of the menstrual cycle for 5 consecutive days. Gonadotropin was injected daily starting on the 5th day post Letrozole administration until human chorionic gonadotropin (HCG) injection day. 250 μg of recombinant HMGα (Merck Serono) was administered when three leading follicles reached 17 mm in diameter or a single dominant follicle reached 18 mm in diameter to trigger ovulation. Ultrasound-guided transvaginal retrieval of oocytes was performed after 36–38 h of the trigger. Oocytes were cryopreserved or in vitro fertilized according to the personal preferences of the patients or their marital status. The oocytes from unmarried patients were frozen for prophylactic fertility preservation. The granulosa cells were removed 2–3 h after the oocytes were collected. The mature MII oocytes were frozen by vitrification. Oocytes from married patients were in vitro fertilized by routing fertilization protocol or Intracytoplasmic sperm injection (ICSI) according to the status of the male semen. Fertilized oocytes were cultured in G1 medium (Vitrolife, Sweden) for 3 days for cleavage stage or 5 days for blastocyst stage. Fresh or freeze-thaw embryos transfer were performed according to the needs of patients.

2.3. Statistical analysis

Clinical outcomes were evaluated by analyzing antral follicle count (AFC), the ratio of FSH/LH, oocyte retrieval number, 2 pronucleus(2 PN) fertilization rate, high-quality embryo rate, clinical pregnancy rate and delivery outcome. The measurement data that passed the normality tests were analyzed by using one-way ANOVA and presented as mean ± standard deviation; The data that did not pass the normality test were analyzed by using a Kruskal–Wallis test and presented as median and interquartile distance. P value < 0.05 was considered to be statistically significant. SPSS software (version 20.0) was used for conducting statistical analysis.

3. Results

Baseline characteristics of patients were shown in Table 1 there was no significant difference of the patients' ages between the three groups, with the average ages all older than 35 years old ($P = 0.619$). There was no significant difference in AFC between the three groups. But the median of FSH/LH in the surgical treatment group is lower than that in the combined treatment group and the control group ($P = 0.008$).

When comparing the IVF outcomes including the number of oocytes retrieval, MII oocytes, 2 PN fertilization rate, the number and the rate of high-quality embryos between the three groups, no statistically significant difference was found (Table 2). The number of oocytes retrieval was 4, 3.5, and 4 in the combined treatment group, the surgery only group, and the control group, respectively, with no significant difference

Table 2
Comparison of the IVF outcomes in each group.

	Combined treatment group	Surgery only group	Control group	*P*
the number of oocytes retrieval [median (IQR)]	4(1.5,9.5)	3.5(1.5,14.5)	4(3,6)	0.922
MII oocytes [median (IQR)]	3(1.5,9)	3.5(1.75,11.5)	4(3,6)	0.868
2 PN fertilization rate[a] (%)	70.32	72.63	56.81	0.318
the number of high-quality embryos [median (IQR)][b]	2(0,4.5)	2(0,2.5)	2(0,3)	0.910
the rate of high-quality embryos[c] (%)	43.40	32.58	44.67	0.903

[a] 2 PN fertilization rate: 2 PN embryos/IVF or ICSI oocytes.
[b] The number of high-quality embryos: the number of high-quality embryos obtained. The standard of high-quality embryos: when the fertilized ooycte is cleaved into 4–8 cell stages, the embryo score is given. The standard of grade I-II embryos (that is, high-quality embryos) is: Grade I embryos: embryos have uniform size and regular morphology, Fragmentation rate in embryo cytoplasm ≤10%; Grade II embryo: The size of blastomeres is uniform or slightly uneven, fragmentation rate in embryo cytoplasm >10% and ≤25%.
[c] The rate of high-quality embryo: the number of high-quality embryo/the number of normal fertilized cleavage embryo *100%[6]

Table 1
Comparison of the basic condition of patients.

	Combined treatment group	Surgery only group	Control group	*P*
Patients number(n)	9	11	14	
Cycles[a](n)	17	14	17	
Cycle cancellation rate n(%)	4(23.5)	2(14)	6(35)	
Age(year)	36.17 ± 4.79	35.71 ± 6.42	37.4 ± 3.82	0.619
FSH/LH[b][median (IQR)]	2.47[a](1.68,4.11) [ab]P = 0.007	1.32[b](0.95,2.16) [bc]P = 0.006	2.58[c](1.73,5.03) [ac]P = 0.608	0.008
AFC[median (IQR)]	8(6,10.75)	9(5.5,14)	5(3,8)	0.101

[a] For ovulation promotion cycle.
[b] FSH/LH: the ratio of Follicle Stimulating Hormone to Luteinizing Hormone.

Table 3
The clinical outcomes of fresh or freeze-thawed embryo transfer cycles in different groups.

		Combined treatment group	Surgery only group	Control group
Total cycles		17	14	17
Frozen oocytes presservation		0	5	0
Frozen embryo presservation		3	5	2
Frozen embryo transfer after thawing	Number of transfer	3	0	0
	Number of deliveries	1	0	0
Fresh embryo transfer	Number of transfer	3	1	9
	Number of deliveries	3	1	1

between groups ($P = 0.922$); The MII numbers were 3, 3.5, and 4, respectively, without statistically significant difference ($P = 0.868$); The number (rate) of high-quality embryos were 2 (43.40%), 2 (32.58%), and 2 (44.67%), respectively, without statistically significant difference, $P = 0.910$ ($P = 0.903$); The fertilization rate of 2 PN was 70.32%, 72.63%, 56.81%, respectively, and no statistically significant difference between the three groups ($P = 0.318$).

The clinical outcomes of fresh or freeze-thawed embryo transfer cycles in different groups were shown in Table 3. In the combined treatment group, 3 fresh embryo transfer cycles resulted in 3 delivers, and 3 freeze-thawed embryo transfer cycles, 1 delivery of a healthy baby. In the surgery only group, 1 case of fresh embryo transfer resulted in one delivery. In the control group, 9 cycles of fresh embryo transfer resulted in 1 delivery.

No recurrence of breast cancer was found during assisted reproductive therapy.

4. Discussion

With the advance of cancer therapy, the long-term survival rate of breast cancer patients has improved. The combination of surgery with adjuvant radiotherapy, chemotherapy, and endocrine therapy has significantly improved the cancer-free and overall survival time of young breast cancer patients. With the longer survival time, short-term and long-term adverse effects of these treatments, including ovarian function damage, have also emerged. A population-based study showed that the pregnancy rate of breast cancer patients was only 3%, which was 40% lower than that of the general population [4], suggesting that fertility preservation is necessary for young breast cancer patients.

In terms of the ovarian function in patients received different cancer therapies, this study found that the combined treatment significantly lowered the ratio of FSH/LH compared to the surgery only group and the control group. This result indicated that adjuvant therapy (endocrine therapy with radiotherapy or chemotherapy) had resulted in the damage of ovarian endocrine function. Therefore, it further indicated that the best time to preserve fertility for breast cancer patients is just after the surgery but before the initiation of adjuvant treatment [9]. A prospective phase 2 neoadjuvant chemotherapy(NACT) study in patients under 43 years of age with stage II and stage III breast cancer at the University of California San Francisco (UCSF) showed that the average time from first diagnosis to initiation of treatment was 40 days for all patients, and there was no significant difference in the average time between patients receiving ovarian stimulation and patients without ovarian stimulation [10]. Therefore, we can make full use of this time-limited window to give patients fertility counseling to make it possible to choose a preventive fertility preservation program as soon as possible.

For breast cancer patients, cryopreservation of embryos has been widely accepted as an effective method for fertility preservation at present. The success rate of embryo cryopreservation is relatively stable. The success rate of live birth from the cryopreserved embryos before the initiation of chemotherapy and/or radiotherapy is similar to that of the age-matched control group [11,12]. Oocyte cryopreservation is suitable for female patients with malignant tumors who are not ready for childbearing or unable to choose embryo cryopreservation for other reasons. Among the breast cancer patients in this study, 4 unmarried patients received oocyte cryopreservation and 10 married patients received embryo cryopreservation.

In terms of the ovarian stimulation protocol, combined usage of aromatase inhibitors and gonadotropins can reduce serum estradiol concentration and in situ estrogen production in the tumor. Therefore, the potential adverse effects of elevated estrogen on breast cancer recurrence can be avoided because breast cancer is a hormone-dependent disease [13]. The data in this study showed that ovarian stimulation with aromatase inhibitors in combination with gonadotropin in breast cancer patients had similar ART outcomes, evaluated by the number of oocytes, 2 PN fertilization rate and the high-quality embryo rate, as that in the non-breast cancer patients with normal ovarian function. This indicated that the combination of aromatase inhibitors and gonadotropin is effective for ovarian stimulation of breast cancer patients.

Fertility preservation after breast cancer diagnosis is currently considered a safe option. Balayla et al. have shown, in a retrospective study, that fertility preservation has no adverse effect even in estrogen receptor-positive breast cancer patients [14]. Moreover, Dabrosin et al. have shown that postoperative breast cancer pregnancy and breastfeeding are safe for women with a lower risk of breast cancer recurrence [15]. In addition, some studies have shown that pregnancy is good for survival outcomes among breast cancer patients who have had recurrence. They found that those who are pregnant may be healthier and less likely to relapse than those who are not pregnant [16]. Consistent with these studies, no relapses have been found in all the patients by the end of the study.

Regarding the safety of aromatase inhibitors for ovarian stimulation, Kim et al. showed that letrozole did not affect the recurrence-free survival rate of breast cancer patients after an average of 5.5 years of follow-up. In a subgroup of this study, even *BRCA* mutation, tumor resection, and tumor ER status did not have impacts on the recurrence-free survival rate [17]. Supporting these findings, Ferreiro group did not find negative effect of aromatase inhibitors on the cancer recurrence or mortality increase [18]. In addition, aromatase inhibitors are safe for live birth. No birth defects have been found in pregnancy after using aromatase inhibitors [18].

We recognize the limitations of this study. The main limitation of this study is the total number of cases of postoperative breast cancer fertility preservation and assisted pregnancy is limited. We have more breast cancer patients who have received postoperative fertility preservation but are still in the process of breast cancer treatment. Once these patients finish their ART cycles, we will have more cases and embryos transfer cycles. Further study with Long term and large sample size should be explored to evaluate the clinical outcomes of fertility preservation.

In conclusion, this study found that adjuvant radiotherapy and chemotherapy following breast cancer surgery can affect ovarian function adversely. The “time-limited window " for better fertility preservation is after surgery and before the initiation of adjuvant treatment. The aromatase inhibitors - based ovarian micro-simulation protocol is effective and safe for assisted reproduction for infertile patients with breast cancer history. This study is of value for oncologists, reproductive doctors, and tumor patients to coordinate and develop safe fertility preservation plans so as to reduce the risk of infertility for breast cancer women.

Author’s roles

The authors’ responsibilities were as follows - X.L.S. and M.M.Z.: collected and analyzed data and drafted the manuscript; C.H.M., X.M.Z. and H.B.C.: collected data and designed research; J.Y.: Performed the oocyte/embryo vitrification and thawing, designed study, and critically

revised the manuscript; R.L., X.W.Z and J.Q.: designed the study.

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Declaration of competing interest

None.

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