Ovarian Tissue Cryopreservation in a Patient with Breast Cancer During Pregnancy: A Case Report

Jiaojiao Cheng

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Xiangyan Ruan (✉ ruanxiangyan@ccmu.edu.cn)

Beijing Obstetrics and Gynecology Hospital, Capital Medical University; Research Centre for Women's Health and University Women's Hospital of Tuebingen, University of Tuebingen, Tuebingen, Germany

<https://orcid.org/0000-0001-7777-247X>

Juan Du

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Fengyu Jin

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Yanglu Li

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Xiaowei Liu

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Husheng Wang

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Muqing Gu

Capital Medical University Beijing Obstetrics and Gynecology Hospital

Alfred O. Mueck

Beijing Obstetrics and Gynecology Hospital, Capital Medical University; Research centre for Women's Health and University Women's Hospital of Tuebingen, University of Tuebingen, Tuebingen, Germany

Case report

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Abstract

Background: Fertility preservation using ovarian tissue cryopreservation (OTC) in patients with certain diseases especially needing chemo- or radiotherapy is becoming a routine management in various Western countries. Our hospital is the first and until now the only center in China using this method. The question is controversial, if treatment of breast cancer during pregnancy (PrBC) should be similar like for non-pregnant young patients with breast cancer. To our knowledge this worldwide is the first report using OTC as fertility preservation for PrBC.

Case presentation: During the 29th week of the pregnancy of a 24-year-old woman needle aspiration cytology of a left breast tumor showed cancer cells. The ultrasound revealed BI-RADS 4a grade. Oncologists recommended the termination of pregnancy. Cesarean section at week 32 was performed, and ovarian tissue samples were collected for OTC to preserve fertility and ovarian endocrine function. Twenty-three ovarian cortex slices were slowly programmed cryopreserved. It is estimated that 13000 follicles have been cryopreserved. Breast nodules and sentinel lymph node biopsy suggested invasive micropapillary carcinoma. Neoadjuvant chemotherapy within 1 week after diagnosis was started. After six courses of neoadjuvant chemotherapy, targeted drug therapy, and Goselin acetate, left mastectomy and left axillary lymph node dissection were performed. In total twenty-three times of radiotherapy, eight trastuzumab targeted therapy, and 17 pertuzumab + trastuzumab double targeted therapy was performed after breast cancer surgery. Until now, more than two years after delivery the ovarian function still is good. We do not see any signs of a negative impact of OTC. The injections of goserelin acetate, every 28 days, are planned to last for the next five years. In addition endocrine therapy with anastrozole, started after the breast cancer surgery, also is scheduled for five years.

Conclusion: OTC for fertility preservation in patients with PrBC does not delay breast surgery, radiotherapy, or chemotherapy which is essential for an effective treatment of breast cancer. We assess this method as a promising fertility preservation method, worldwide for the first time now also used in a patient getting breast cancer in pregnancy.

Background

In 2020, there were 2.26 million new cases of female breast cancer worldwide, far exceeding other types of female cancers, accounting for about 24.5% of female cancers ^[1]. Breast cancer during pregnancy (PrBC) occurring as primary breast cancer diagnosed during pregnancy ^[2], is accounting for about 4% of breast cancer cases in women under the age of 45 ^[3]. The incidence of PrBC is estimated to be about 1 in 3000 pregnancies ^[3]. Breast cancer during the postpartum period (PPBC) arising within 5–10 years after delivery, is accounting for an estimated 35–55% of all breast cancer cases in women under 45 years of age ^[4]. The incidence of pregnancy-related breast cancer has increased significantly in the past ten years ^[5], especially in developed countries. It may be related to the postponement of the age of first pregnancy and the continued increase in the incidence of young breast cancer ^[6]. With the development of early diagnosis and treatment strategies of breast cancer, the disease-free and overall survival rate of patients

with breast cancer have been greatly improved [7]. However, compared with normal women, the fertility rate of breast cancer patients decreased significantly about 40–67% after diagnosis and treatment. More and more attention has been paid to the effects of breast cancer chemotherapy, radiotherapy, and endocrine therapy on the quality of life and fertility of breast cancer patients [8].

At present, fertility preservation strategies mainly include embryo and oocyte cryopreservation, ovarian tissue cryopreservation (OTC), in vitro maturation (IVM), and gonadotropin-releasing hormone analog (GnRHa) therapy during chemotherapy [9]. However, to our knowledge there are worldwide no reports about OTC in patients with PrBC. This report is a case of performing OTC in a young patient with PrBC, which could provide new understanding for clinicians and first evidence for fertility preservation in patients with PrBC.

Case Presentation

A 26-year old Chinese lady two years ago had a pregnancy. At week 15 of gestation she touched a solid tumor in her left breast. Physical examination showed that the left breast mass was the size of a peanut, with milk overflow, no tenderness, and no apparent depression or bumped on the breast surface. Breast ultrasound showed hyperplasia of mammary glands, and low echo of the left breast, 12*8mm, 11*7mm, breast imaging reporting and data system (BI-RADS) 4a grade. The oncologist suggested reexamination after three months. The patient realized that the breast mass gradually increased, so she saw a doctor again in a large grade 3A hospital. Needle aspiration cytology of the left breast tumor was performed on March 14, 2019, and cancer cells were detected. Using breast ultrasound, a solid nodule was seen in the lower left breast quadrant, 18*13mm, clear boundary, lobulated, BI-RADS 3 grade. A solid cystic nodule, 10*8mm, was found, and a solid area could be seen in it, range about 4*3mm, BI-RADS 4a. Oncologists recommended that the patient should terminate the pregnancy.

Cesarean section was performed at 32 weeks and four days of pregnancy in the Beijing Obstetrics and Gynecology Hospital, Capital Medical University. The boy is healthy by now. At the same time of the cesarean section, the ovarian tissue biopsy was taken for OTC to preserve fertility and ovarian function. The amount of ovarian tissue taken from one side was 1/2, from the other was 1/3 of the ovary. The condition of fresh ovarian tissue was good, with a corpus luteum and rich blood vessels.

The ovarian tissue was successfully processed and slow-programmatically cryopreserved in ovarian tissue cryobank, and fresh cortex viability and morphology assessment was performed. The methods description was consistent with the previously published article [10, 11]. A total of 23 pieces of the ovarian cortex were frozen. The number of follicles in a round cortical piece of 2 mm is about 45, and it is estimated that 13000 follicles have been frozen for the patient. The images of follicular viability are shown in Fig. 1, and the images of HE staining in the cortex are shown in Fig. 2.

A biopsy of the left breast tumor was performed on April 18, 2019. Pathological findings showed that (breast tissue) invasive micropapillary carcinoma of the breast, immunohistochemical results: estrogen

receptor (ER) (weak-medium positive, 10%), progesterone receptor (PR) (-), androgen receptor (AR) (weak-medium positive, 80%), Human epidermal growth factor receptor 2 (HER-2) (3 +). Ki-67 (index 30%), p53 (+), Cytokeratin 14 (CK14) (-), D2-40 (-), Epithelial membrane antigen (EMA) (+), CK5/6 (-), Epidermal growth factor receptor (EGFR) (-), Synaptophysin (Syn) (-), chromogranin A (CgA) (-). A left axillary sentinel lymph node biopsy was performed on April 24, 2019. Pathology showed that the metastatic carcinoma of the lymph node (left sentinel) (2 positives in 3) and the isolated tumor cells could be seen in the capsule of the other lymph node.

Neoadjuvant chemotherapy before breast surgery was given. The first chemotherapy regimen was docetaxel 120mg, epirubicin 110mg, and cyclophosphamide 900mg, q3w. Starting after the first course of chemotherapy, injections of goserelin acetate every 28 days, 3.6mg, were given, planned to continue for five years. There was no menstruation until now from the beginning of chemotherapy. According to the results of immunohistochemistry, the 2nd-6th chemotherapy regimen was adjusted as follows: docetaxel 110mg and carboplatin 500mg. Targeted therapy, trastuzumab, first dose was 17ml, then changed to 13ml. A total of 6 times of targeted therapy were performed before breast surgery.

Ultrasonographic examination of breast and axillary lymph nodes before breast surgery showed that low echo was seen at the edge of the gland in the direction of 6–7 o'clock in the left breast, 19*16*8mm. The boundary was unclear, and dotted strong echo was diffused in the left breast, BI-RADS 6.

Simple left mastectomy (preserving nipple-areola) and left axillary lymph node dissection were performed on September 10, 2019. Left breast reconstruction + acellular allogenic dermis implantation + dilator implantation was performed after breast surgery. Pathological report: In (left breast) breast tissue could be seen few invasive carcinomas (the largest 7mm), and a vascular tumor thrombus, the bottom cutting edge not special, lymph nodes showing chronic inflammation (left axilla 0/18). Immunohistochemical results showed: ER (weak positive, 10%), PR (-), AR (weak positive, 90%), Her-2 (3 +), Ki-67 (index 25%), p53 (scattered +), EGFR (-), CK14 (-), CK5/6 (-), p63 (-), CgA (-), Syn (-), EMA (+).

After breast surgery, fixed-field intensity-modulated radiation therapy was performed with a 6MV-X line. 95% of the plan clinical tumor volume included left upper and lower clavicle, chest wall, dose 46Gy/23 times (2Gy/f), 5 f / w, filler bolus 0.5cm.

Fourteen courses of trastuzumab after surgery, and then pertuzumab + trastuzumab 13ml double targeted therapy for one year were performed, about 17 courses. After breast surgery, the injection of goserelin acetate was continued every 28 days, which is planned to last for five years. The endocrine therapy using anastrozole, a potent aromatase inhibitor, is scheduled to last also for five years.

The serum hormone levels before OTC and one year, 1.5 years, 2 years, and 2.2 years after OTC are shown in Table 1. At present, it seems that the AMH level of the patients is in the normal range, and the levels of FSH, LH, and E2 are all at a low level.

Table 1
Hormone levels of before and after cryopreservation

	2019.04.08 (before OTC)	2020.05.08 (1 year after OTC)	2020.10.12 (1.5 years after OTC)	2021.03.17 (2 years after OTC)	2021.06.18 (2.2 years after OTC)
AMH (ng/ml)	2.73	1.18	1.3	2.54	3.70
FSH (IU/L)	0	4.05	6.08	3.66	4.64
LH (IU/L)	0	1.46	1.31	1.03	0.39
E2 (pg/ml)	15000	11.8	11.8	33.67	19.37
AMH: anti-müllerian hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol; OTC: ovarian tissue cryopreservation					

Discussion And Conclusion

A 26-year-old woman more than two years ago was diagnosed with PrBC in the third trimester and underwent cesarean section at 32 weeks and four days to terminate the pregnancy. Subsequently, the patient underwent chemotherapy, radiotherapy, targeted therapy, breast cancer surgery, and long-term endocrine therapy. During cesarean section ovarian tissue samples were collected and OTC was performed to preserve fertility and ovarian function, without additional ovarian tissue biopsy surgery. This technique for fertility preservation avoids ovarian hyper-stimulation, and there is no need to delay follow-up anti-cancer treatment. For this patient the risk of premature ovarian insufficiency (POI) in the future is high, but her survival rate is very high. Only one child was born, and the patient still is young and wants to preserve ovarian function and fertility.

PrBC should be regarded as an independent entity different from breast cancer occurring during the postpartum period (PPBC). The treatment of PrBC is individualized according to gestational age and considers the fetus's safety. The treatment of PPBC does not need to consider these issues. The histopathological and immunohistochemical results of tumors in PrBC patients seem to be similar to those in young non-pregnant breast cancer patients. High estrogen and progesterone in pregnancy may stimulate the proliferation of breast cancer cells in PrBC patients. However, the prognosis does not seem to differ from non-pregnant patients of the same age and stage^[3,4]. As with the patients in this study, the current outcome is good. Therefore, the biological characteristics of the tumor are more likely to be determined by the age at the time of diagnosis than by pregnancy.

In 2013, the American Society of Clinical Oncology conducted a comparative study of 311 patients with PrBC and 865 patients with non-pregnancy-related breast cancer^[12]. It was found that the overall survival rate of patients with PrBC was similar to that of patients with non-pregnancy-related breast cancer. This information is essential when the PrBC patient is consulted and supports the start of treatment while pregnancy can continue. For our case according to the recommendations of the oncologists the

pregnancy after confirmation of the diagnosis was terminated, i.e. anti-cancer treatment was not started during pregnancy.

The 5-year relative survival rate of breast cancer patients is about 90% ^[13]. More than 50% of young women with breast cancer want to become pregnant after treatment ^[14]. It is reported that their chances of pregnancy are 40–67% lower than that of the general population ^[15]. Some studies have reported that the live birth rate of breast cancer after treatment is less than 5% ^[16, 17]. Recently, a large sample study showed that in breast cancer patients with and without fertility preservation, the cumulative incidence of live births after five years of breast cancer diagnosis was 19.4% and 8.6%, respectively, 40.7% and 15.8% ten years later ^[18]. The research on pregnancy safety after breast cancer treatment is complex, and randomized controlled trials are impossible, so the evidence to guide clinical practice is limited.

The best time to conceive after the diagnosis of breast cancer is still inconclusive. The main concern is the recurrence of cancer and the interruption of endocrine therapy. ER-negative patients should be delayed for 2–3 years according to the prognosis. Positive patients can discuss whether to discontinue endocrine treatment after three years, but patients must be informed of the lack of data support ^[19]. One large meta-analysis ^[20] found that post-breast cancer pregnancy had no adverse effect on survival. Women who became pregnant after breast cancer had even higher survival rates than non-pregnant breast cancer patients ^[21].

Patients with PrBC received chemotherapy during pregnancy and post-natal chemotherapy, and an observational study reported no difference in survival rate ^[22]. Chemotherapy during pregnancy is generally carried out in the third trimester of pregnancy, and there is no increase in the rate of congenital malformation. The available data confirm that the mother and fetus are safe in breast cancer treatment during pregnancy ^[22]. Because preterm delivery is closely related to adverse events, full-term delivery seems to be the most important.

The degree of ovarian function damage caused by breast cancer chemotherapeutic drugs is related to the patient's age, chemotherapy type, dose, and duration ^[23]. Among the commonly used chemotherapeutic drugs, alkylating agents have the strongest gonadal toxicity, followed by platinum, paclitaxel, anthracycline, and so on. Some patients may have temporary or permanent amenorrhea during chemotherapy. 40–60% of women under 40 years old will have amenorrhea, and more than 80% of women over 40 years old will have amenorrhea. Although some patients' menstruation can recover after chemotherapy, ovarian function is still impaired. This suggests that menstruation does not necessarily mean giving birth ^[24, 25]. It is recommended that fertility preservation strategies should be taken as far as possible for patients who still have fertility wishes in the future before the start of chemotherapy ^[26].

Radiotherapy is an essential part of comprehensive breast cancer treatment. It is an important measure to reduce the recurrence and prolong the survival of patients undergoing breast-conserving surgery and high-risk mastectomy ^[27]. In breast cancer patients receiving standard whole breast radiotherapy, 2.1–7.6

cGy (1Gy = 100cGy) reaches the uterus and ovaries through the internal scattering of 50Gy radiation dose to the breast^[28]. Radiotherapy is not recommended for PrBC, but can be chosen according to the condition after stopping breastfeeding at the end of pregnancy^[29].

Endocrine therapy refers to drugs to block the promoting effect of sex hormones on breast cancer cells according to the expression of ER and PR in breast cancer tissue^[30]. Anastrozole is a potent, selective aromatase inhibitor of triazole, blocking estrogen biosynthesis by inhibiting the aromatase^[31]. Estrogen is the main factor that stimulates the growth of breast cancer cells. Although endocrine therapy has no reproductive toxicity, endocrine treatment lasts for 5–10 years, and the ovarian function of patients continues to decrease with age. Therefore, breast cancer patients with fertility needs are recommended fertility preservation before endocrine therapy^[7].

Embryo cryopreservation is the most widely used and technically perfect fertility preservation strategy in the clinic, suitable for married women after puberty^[32]. Two aspects are worth paying attention to 1) due to a series of operations such as ovulation stimulation, in vitro fertilization, and embryo cryopreservation, the treatment of breast cancer may be delayed for about two weeks; 2) the hyper-physiological dose of estrogen caused by ovulation stimulation maybe promote the development of breast cancer. There has been a report^[33] of fertility preservation with random-start controlled ovarian stimulation and embryo cryopreservation for early pregnancy-associated breast cancer.

Oocyte cryopreservation also is suitable for unmarried women after puberty. It is recommended that women < 38 years old freeze 15–20 MII stage oocytes, the chance of at least one live birth is 70–80%. For 38-40-year-old women, if 25–30 MII stage oocytes are frozen, the chance of at least one live birth is 65–75%^[34]. IVM can reduce ovarian stimulation, avoid a high estrogen state, and be combined with OTC^[35]. Although it is estimated that more than 5000 babies have been born through IVM technology worldwide^[36], IVM is still considered an experimental technology by the American Society of Reproductive Medicine (ASRM)^[37].

The OTC technique requires minimally invasive surgery to take part in the ovarian tissue before gonadotoxicity treatment, without the need of ovarian stimulation to obtain oocytes. It is the only fertility preservation method for patients who cannot delay anti-cancer treatment^[38, 39]. More than 200 babies have been born worldwide through OTC and transplantation^[40], and in 2019 the ASRM pointed out that this technique is no longer experimental^[41]. The ovarian tissue cryobank of Beijing Obstetrics and Gynecology Hospital, the first and until now the only one in China^[42], has up today successfully cryopreserved more than 300 cases of ovarian tissue, of which 10.8% are breast cancer patients. A total of 10 cases of ovarian tissue were transplanted^[11, 43], including 1 case of breast cancer with negative ER, PR, and HER2 (3 +). The ovarian function was recovered after transplantation. One case of myelodysplastic syndrome (MDS) successfully became pregnant naturally after ovarian transplantation and is currently still in pregnancy (publication accepted).

Breast cancer is one of the most common indications for OTC and transplantation. In five major centers in Europe, of 285 transplant patients, 96 were breast cancer patients, 7 relapsed (7.3%) [44]. Breast cancer itself is a known disease with a risk of recurrence [45]. At the time of breast cancer diagnosis, the young age is associated with an increased risk of recurrence [46], while patients who receive OTC are usually very young, almost all under the age of 40. All relapses were dependent on the primary disease and had nothing to do with ovarian tissue transplantation, because all relapses were far away from the transplant site, and most of them were close to the location of primary cancer. The recurrence rate of 7.3% is similar to that of breast cancer women under 40 years old observed in the literature, with a local recurrence rate of 10% [47] and a 10-year recurrence rate of 4-8.7% [45]. Regarding transplanting ovarian tissue from breast cancer patients, it should be kept in mind that they are still cancer patients in remission.

Conclusion

Ovarian tissue cryopreservation for fertility preservation in patients with PrBC does not delay breast surgery, radiotherapy, or chemotherapy which is essential for an effective treatment of breast cancer. Until now, more than two years after delivery, we do not see any signs of a negative impact performing OTC simultaneously with cesarean section, and thereafter getting chemotherapy, radiotherapy, targeted therapy, breast cancer surgery, and endocrine therapy. At present, the ovarian function is still good. Although long-term results and especially the results after retransplantation of ovarian tissue still are missing, we assess this method, to date in China only performed in our hospital, as a promising fertility preservation method, worldwide for the first time now also used in a patient getting breast cancer in pregnancy.

Abbreviations

OTC

ovarian tissue cryopreservation

POI

premature ovarian insufficiency

PrBC

breast cancer occurs during pregnancy

PPBC

breast cancer occurs during the postpartum period

IVM

in vitro maturation

GnRHa

gonadotropin-releasing hormone analog

BI-RADS

breast imaging reporting and data system

ER

estrogen receptor

PR

progesterone receptor

AR

androgen receptor

HER-2

human epidermal growth factor receptor 2

EMA

epithelial membrane antigen

EGFR

epidermal growth factor receptor

Syn

synaptophysin

CgA

chromogranin A

ASRM

American society of reproductive medicine

FSH

follicle-stimulating hormone

LH

luteinizing hormone

AMH

anti-müllerian hormone

E2

estradiol

MDS

myelodysplastic syndrome

Declarations

Ethics approval and consent to participate

This study was approved by the Beijing Obstetrics and Gynecology Hospital, Capital Medical University, China ethics committee on 15th March 2017 (Protocol number 2017-KY-020-01).

Consent for publication

The patient provided written informed consent for the publication of this case report.

Availability of data and materials

All the generated data are included in this article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JC wrote the original draft and revised the manuscript.

XR acquired the medical report and data from the patient, project, and funds leader.

JD, FJ, and YL cryopreserved the ovarian tissue.

MG cryopreserved the ovarian tissue and follow-up patients.

XL has done a cesarean section and ovarian tissue biopsy.

HW transported the ovarian tissue to cryobank.

AM guided the implementation of the project and revised the final manuscript.

All authors read and approved the final paper.

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Figures

Figure 1

Typical pictures of follicles in the fresh ovarian cortex. a1 is the image under the fluorescence microscope, and a2 is the image under the optical microscope.

Figure 2

Typical HE staining images of follicles in the fresh ovarian cortex. The black arrow refers to the follicle. In a1, bar=100μm; in a2, bar=50μm