Case report

Keywords: Breast cancer during pregnancy, ovarian tissue cryopreservation, fertility preservation, ovarian function

DOI: https://doi.org/10.21203/rs.3.rs-824318/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
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 (index25%), 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 insucion (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\textsuperscript{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\textsuperscript{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% \cite{13}. More than 50% of young women with breast cancer want to become pregnant after treatment \cite{14}. It is reported that their chances of pregnancy are 40–67% lower than that of the general population \cite{15}. Some studies have reported that the live birth rate of breast cancer after treatment is less than 5% \cite{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 \cite{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 \cite{19}. One large meta-analysis \cite{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 \cite{21}.

Patients with PrBC received chemotherapy during pregnancy and post-natal chemotherapy, and an observational study reported no difference in survival rate \cite{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 \cite{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 \cite{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 suggest, that menstruation does not necessarily mean giving birth \cite{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 \cite{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 \cite{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\(^\text{[28]}\). Radiotherapy is not recommended for PrBC, but can be chosen according to the condition after stopping breastfeeding at the end of pregnancy\(^\text{[29]}\).

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\(^\text{[30]}\). Anastrozole is a potent, selective aromatase inhibitor of triazole, blocking estrogen biosynthesis by inhibiting the aromatase\(^\text{[31]}\). 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\(^\text{[7]}\).

Embryo cryopreservation is the most widely used and technically perfect fertility preservation strategy in the clinic, suitable for married women after puberty\(^\text{[32]}\). 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\(^\text{[33]}\) 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%\(^\text{[34]}\). IVM can reduce ovarian stimulation, avoid a high estrogen state, and be combined with OTC\(^\text{[35]}\). Although it is estimated that more than 5000 babies have been born through IVM technology worldwide\(^\text{[36]}\), IVM is still considered an experimental technology by the American Society of Reproductive Medicine (ASRM)\(^\text{[37]}\).

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\(^\text{[38, 39]}\). More than 200 babies have been born worldwide through OTC and transplantation\(^\text{[40]}\), and in 2019 the ASRM pointed out that this technique is no longer experimental\(^\text{[41]}\). The ovarian tissue cryobank of Beijing Obstetrics and Gynecology Hospital, the first and until now the only one in China\(^\text{[42]}\), 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\(^\text{[11, 43]}\), 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%)\textsuperscript{[44]}. Breast cancer itself is a known disease with a risk of recurrence\textsuperscript{[45]}. At the time of breast cancer diagnosis, the young age is associated with an increased risk of recurrence\textsuperscript{[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%\textsuperscript{[47]} and a 10-year recurrence rate of 4-8.7%\textsuperscript{[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
epimelial 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.

Funding: This study was supported by Natural Science Foundation of Beijing (7202047); Beijing Capital Foundation for Medical Science Development and Research (2020-2-2112); Beijing Municipal Administration of Hospitals’ Ascent Plan (DFL20181401)

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.

Acknowledgments

For establishing the first ovarian tissue cryobank in China, the authors thank Prof. Markus Montag (International Repro lab Consulting ilabcomm GmbH, FertiProtekt Network, Germany) and Dr. Jana Liebenthron (University Women’s Hospital, Duesseldorf, Germany) for their generous help and continuing support.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.

2. Amant F, Loibl S, Neven P, Calsteren KV. Breast cancer in pregnancy. Lancet. 2012;379(9815):570–9.

3. Loibl S, von Minckwitz G, Gwyn K, Ellis P, Blohmer JU, Schlegelberger B, et al. Breast carcinoma during pregnancy. International recommendations from an expert meeting. Cancer. 2006;106(2):237–46.

4. Amant F, Lefrere H, Borges VF, Cardonick E, Lambertini M, Loibl S, et al. The definition of pregnancy-associated breast cancer is outdated and should no longer be used. Lancet Oncol. 2021;22(6):753–
4. Andersson TM, Johansson A, Hsieh CC, Cnattingius S, Lambe M. Increasing incidence of pregnancy-associated breast cancer in Sweden. Obstet Gynecol. 2009;114(3):568–72.

5. Wang B, Yang Y, Jiang Z, Zhao J, Mao Y, Liu J, et al. Clinicopathological characteristics, diagnosis, and prognosis of pregnancy-associated breast cancer. Thorac Cancer. 2019;10(5):1060–8.

6. Warner E, Glass K, Foong S, Sandwith E. Update on fertility preservation for younger women with breast cancer. CMAJ. 2020;192(35):E1003–9.

8. von Wolff M, Dittrich R, Liebenthron J, Nawroth F, Schüring AN, Bruckner T, et al. Fertility-preservation counselling and treatment for medical reasons: data from a multinational network of over 5000 women. Reprod Biomed Online. 2015;31(5):605–12.

9. Shah NM, Scott DM, Kandagatla P, Moravek MB, Cobain EF, Burness ML, et al. Young Women with Breast Cancer: Fertility Preservation Options and Management of Pregnancy-Associated Breast Cancer. Ann Surg Oncol. 2019;26(5):1214–24.

10. Li Y, Ruan X, Liebenthron J, Montag M, Zhou Q, Kong W, et al. Ovarian tissue cryopreservation for patients with premature ovary insufficiency caused by cancer treatment: optimal protocol. Climacteric. 2019;22(4):383–9.

11. Ruan X, Cheng J, Korell M, Du J, Kong W, Lu D, et al. Ovarian tissue cryopreservation and transplantation prevents iatrogenic premature ovarian insufficiency: first 10 cases in China. Climacteric. 2020;23(6):574–80.

12. Amant F, von Minckwitz G, Han SN, Bontenbal M, Ring AE, Giermek J, et al. Prognosis of women with primary breast cancer diagnosed during pregnancy: results from an international collaborative study. J Clin Oncol. 2013;31(20):2532–9.

13. Coleman MP, Forman D, Bryant H, Butler J, Rachet B, Maringe C, et al. Cancer survival in Australia, Canada, Denmark, Norway, Sweden, and the UK, 1995–2007 (the International Cancer Benchmarking Partnership): an analysis of population-based cancer registry data. Lancet. 2011;377(9760):127–38.

14. Azim HJ, Peccatori FA, de Azambuja E, Piccart MJ. Motherhood after breast cancer: searching for la dolce vita. Expert Rev Anticancer Ther. 2011;11(2):287–98.

15. Gerstl B, Sullivan E, Ives A, Saunders C, Wand H, Anazodo A. Pregnancy Outcomes After a Breast Cancer Diagnosis: A Systematic Review and Meta-analysis. Clin Breast Cancer. 2018;18(1):e79–88.

16. Pagani O, Partridge A, Korde L, Badve S, Bartlett J, Albain K, et al. Pregnancy after breast cancer: if you wish, ma'am. Breast Cancer Res Treat. 2011;129(2):309–17.

17. Lamberti M, Del ML, Pescio MC, Andersen CY, Azim HA Jr, Peccatori FA, et al. Cancer and fertility preservation: international recommendations from an expert meeting. BMC Med. 2016;14:1.

18. Marklund A, Lundberg FE, Eloranta S, et al. Reproductive Outcomes After Breast Cancer in Women With vs Without Fertility Preservation. JAMA Oncol. 2021;7(1):86–91.

19. Margulies AL, Selleret L, Zilberman S, et al. Pregnancy after cancer: for whom and when? Bull Cancer. 2015;102(5):463–9.
20. Azim HJ, Santoro L, Pavlidis N, Hedayati E, Pettersson K, Rodriguez-Wallberg KA. Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. Eur J Cancer. 2011;47(1):74–83.

21. Christinat A, Pagani O. Fertility after breast cancer. Maturitas. 2012;73(3):191–6.

22. Loibl S, Han SN, von Minckwitz G, Bontenbal M, Ring A, Giermek J, et al. Treatment of breast cancer during pregnancy: an observational study. Lancet Oncol. 2012;13(9):887–96.

23. Levine JM, Kelvin JF, Quinn GP, Gracia CR. Infertility in reproductive-age female cancer survivors. Cancer. 2015;121(10):1532–9.

24. Lambertini M, Goldrat O, Clatot F, Demeestere I, Awada A. Controversies about fertility and pregnancy issues in young breast cancer patients: current state of the art. Curr Opin Oncol. 2017;29(4):243–52.

25. Abusief ME, Missmer SA, Ginsburg ES, Weeks JC, Partridge AH. The effects of paclitaxel, dose density, and trastuzumab on treatment-related amenorrhea in premenopausal women with breast cancer. Cancer. 2010;116(4):791–8.

26. Waks AG, Partridge AH. Fertility Preservation in Patients With Breast Cancer: Necessity, Methods, and Safety. J Natl Compr Canc Netw. 2016;14(3):355–63.

27. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. Lancet. 2021;397(10286):1750–69.

28. Bajpai J, Majumdar A, Satwik R, Rohatgi N, Jain V, Gupta D. Practical consensus recommendations on fertility preservation in patients with breast cancer[J]. South Asian J Cancer. 2018;7(2):110–4.

29. Kal HB, Struikmans H. Radiotherapy during pregnancy: fact and fiction. Lancet Oncol. 2005;6(5):328–33.

30. Marti C, Sanchez-Mendez JI. The Present and Future of Neoadjuvant Endocrine Therapy for Breast Cancer Treatment. Cancers (Basel). 2021;13(11):2538.

31. Masuda N, Sagara Y, Kinoshita T, Iwata H, Nakamura S, Yanagita Y, et al. Neoadjuvant anastrozole versus tamoxifen in patients receiving goserelin for premenopausal breast cancer (STAGE): a double-blind, randomised phase 3 trial. Lancet Oncol. 2012;13(4):345–52.

32. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol. 2018;36(19):1994–2001.

33. Pereira N, Kligman I, Hunt R, Kopparam R, Wahmann B, Rosenwaks Z. Fertility preservation with random-start controlled ovarian stimulation and embryo cryopreservation for early pregnancy-associated breast cancer. Gynecol Endocrinol. 2019;35(3):214–6.

34. Doyle JO, Richter KS, Lim J, Stillman RJ, Graham JR, Tucker MJ. Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval. Fertil Steril. 2016;105(2):459–66.
35. Telfer EE, Andersen CY. In vitro growth and maturation of primordial follicles and immature oocytes. Fertil Steril. 2021;115(5):1116–25.

36. Yang ZY, Chian RC. Development of in vitro maturation techniques for clinical applications. Fertil Steril. 2017;108(4):577–84.

37. Practice Committees of the American Society for Reproductive Medicine, the Society of Reproductive Biologists and Technologists, and the Society for Assisted Reproductive Technology. In vitro maturation: a committee opinion. Fertil Steril. 2021;115(2):298–304.

38. Fabbri R, Vicenti R, Magnani V, Pasquinelli G, Macciocca M, Parazza I, et al. Cryopreservation of ovarian tissue in breast cancer patients: 10 years of experience. Future Oncol. 2012;8(12):1613–9.

39. Donnez J, Dolmans MM. Fertility preservation in men and women: Where are we in 2021? Are we rising to the challenge? Fertil Steril. 2021;115(5):1089–90.

40. Dolmans MM, Falcone T, Patrizio P. Importance of patient selection to analyze in vitro fertilization outcome with transplanted cryopreserved ovarian tissue. Fertil Steril. 2020;114(2):279–80.

41. Practice Committee of the American Society for Reproductive Medicine. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril. 2019;112(6):1022–33.

42. Ruan X. Chinese Society of Gynecological Endocrinology affiliated to the International Society of Gynecological Endocrinology Guideline for Ovarian Tissue Cryopreservation and Transplantation. Gynecol Endocrinol. 2018;34(12):1005–10.

43. Ruan X, Du J, Korell M, Kong W, Lu D, Jin F, et al. Case report of the first successful cryopreserved ovarian tissue retransplantation in China. Climacteric. 2018;21(6):613–6.

44. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril. 2021;115(5):1102–15.

45. Plichta JK, Rai U, Tang R, Coopey SB, Buckley JM, Gadd MA, et al. Factors Associated with Recurrence Rates and Long-Term Survival in Women Diagnosed with Breast Cancer Ages 40 and Younger. Ann Surg Oncol. 2016;23(10):3212–20.

46. Arvold ND, Taghian AG, Niemierko A, Raad RFA, Sreedhara M, Nguyen PL, et al. age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol. 2011;29(29):3885–91.

47. Bartelink H, Horiot JC, Poortmans P, Struikmans H, Van den Bogaert W, Barillot I, et al. Recurrence rates after treatment of breast cancer with standard radiotherapy with or without additional radiation. N Engl J Med. 2001;345(19):1378–87.

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.

![Image](image_url)

**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