Embryo Cryopreservation as an Alternative to Treat Infertility in Breast Cancer Survivors in Reproductive Age: Case Reports

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INTRODUCTION

In the last three decades, the mortality rate from BC in several countries has decreased significantly, mainly due to the success of early detection programs and advances in the administration of combination therapy [1]. In combination therapy, chemotherapy has an essential role, and with advances in tissue pathology examination, the chemotherapy selection can be made more precisely. Thus, the role of giving chemotherapy is now becoming increasingly important.

Chemotherapy has also significantly reduced breast cancer (BC) mortality. However, several types of chemotherapy drugs affect the reproductive system. One of the side effects of chemotherapy on the reproductive system is gonadotoxic, causing infertility [2,3].

Specifically, based on the Surabaya Oncology Hospital records from 2014–2019, it is known that BC diagnosed at reproductive age has almost reached half the population. A total of 39.3% of patients received neoadjuvant, adjuvant, or palliative chemotherapy at the Surabaya Oncology Hospital. Meanwhile, approximately 61.97% of those patients received chemotherapy regimens causing a moderate to high risk of leading to infertility.

On the other side, several fertility preservation options are currently available to treat infertility. The most common fertility preservation option is embryo or oocyte cryopreservation, which can be performed before starting chemotherapy. Ovarian stimulation followed by in vitro fertilization (IVF) and embryo cryopreservation is presently the most established method [3]. Nevertheless, using IVF and embryo cryopreservation in BC patients raises dilemmas regarding efficacy and safety.

Here, we reported two BC patients who had surgery, underwent pre-chemotherapy fertility preservation, received chemotherapy and hormonal therapy, and became pregnant after the IVF program. After experiencing one cycle of IVF, both women gave birth to healthy babies.

This paper, therefore, aims to report the cases of safe pregnancy in BC survivors through pre-chemotherapy IVF followed by embryo cryopreservation. To our knowledge, this is the first report of safe pregnancy in BC patients through pre-chemotherapy IVF, followed by embryo cryopreservation in our region.
CASE PRESENTATION

Case 1
A 35-year-old woman came to the Surgical Clinic of the Surabaya Oncology Hospital on June 17, 2015, with complaints of a lump in the right breast for two weeks. The patient has been married for five years and has no children. Then, a physical examination revealed a 3 cm diameter mass in the outer quadrant of the right breast. Breast and axilla ultrasound also found highly suggestive of a malignant mass in the right breast and lymphadenopathy in the right axilla (Figure 1). In addition, confirmation by fine needle aspiration biopsy of the right breast revealed ductal carcinoma. The patient’s diagnosis was right BC. On June 22, 2015, she underwent a simple mastectomy and latissimus dorsi (LD) flap reconstruction. The histopathology and immunohistochemistry examination showed invasive carcinoma of no particular type with pathological grade III, stage T2pN0M0, and stage IIIA with positive estrogen (5%), progesterone receptor (5%), and Her-2/Neu (3+) status.

Figure 1. (A) Right breast ultrasound image (Asterisk showing highly suggestive of malignant mass); (B) Right axilla ultrasound image (Asterisk showing lymphadenopathy)

The patient was planned for adjuvant chemotherapy and hormonal therapy. Regarding the patient’s desire to have children, the patient underwent an IVF procedure before undergoing chemotherapy. A series of IVF procedures were then carried out. The patient started controlled ovarian stimulation on July 28, 2015, with 112.5 IU recombinant follicle-stimulating hormone (r-FSH) for 11 days. The r-FSH dose was adjusted every two until four days depending on the ultrasound and estradiol results. On August 8, 2015, eight follicles developed on the left ovary with the largest diameter of 22 mm, and seven developed on the right with the largest diameter of 17 mm (Figure 2). Recombinant human chorionic gonadotropin (r-hCG) was also used to induce ovulation on the same day. On August 10, 2015, oocyte retrieval was carried out, with the results of 11 oocytes retrieved. On the same day, Intracytoplasmic Sperm Injection (ICSI) was used to fertilize all oocytes. On August 11, 2015, nine oocytes were fertilized, and nine embryos were developed (grade A embryos) in the next three days. On August 14, 2015, all nine embryos were cryopreserved with the vitrification method.

On August 15, 2015, the patient immediately began receiving adjuvant chemotherapy with a cyclophosphamide-doxorubicin-5-fluorouracil (CAF) regimen every 21 days for up to six cycles. The last cycle was given on November 28, 2015. On December 30, 2015, tamoxifen 20 mg once a day was given for a year. The patient stopped taking tamoxifen in December 2016 due to her desire for pregnancy, considering that this drug could hinder correct embryo implantation.

On December 29, 2016, the patient’s endometrium was prepared for embryo transfer and pregnancy with an artificial cycle before embryo transfer. She started the treatment with estradiol valerate 2 mg for 16 days. After 16 days, ultrasound and estradiol levels were measured, and the treatment continued with estradiol valerate 2 mg and progesterone 400 mg. Four frozen embryos were thawed and cultured in a CO2 incubator the day before embryo transfer. Embryo transfer was performed on January 17, 2017, and three (grade-A embryos) were transferred. On January 27, 2017, the biochemical pregnancy test result was positive (human chorionic gonadotropin was 144 mIU/mL). On February 17, 2017, the patient underwent the first gestational ultrasound showing a gestational sac and fetal pole. At eight weeks of pregnancy, fetal heartbeat was positive, so the patient was referred to the obstetricians. Antenatal care made by the obstetricians showed normal development of pregnancy.

Figure 2. Transvaginal ultrasound showing follicles in the right and left ovary
On September 13, 2017, the pregnancy was terminated with a Caesarean section at week 37 due to gestational hypertension. A healthy boy was born with normal birth weight and length. The post-partum phase was normal, but exclusive breastfeeding could not be done due to a lack of milk production. Three months after giving birth, in December 2017, the patient continued to take tamoxifen. The last follow-up was in April 2022, finding that she had a healthy child, no complaints, and no evidence of disease. The chronology overview of this case is shown in Figure 3.

Case 2

A 29-year-old woman came to the Surabaya Oncology Hospital on May 6, 2014, with a chief complaint of a lump in her left breast for a week. The patient has been married for five years and has no children. On physical examination, there was a mass in the retro nipple and lower outer quadrant. A breast ultrasound also found two irregular solid lesions with microcalcifications inside the left breast suspected of malignant mass and a solid lobulated lesion in the left breast (Figure 4). Besides, confirmation by fine needle aspiration biopsy of the right breast revealed ductal carcinoma. As such, the patient’s diagnosis was left BC. On May 9, 2014, the patient underwent a simple mastectomy and LD flap reconstruction, and the histopathologic and immunohistochemistry examination showed invasive pleomorphic lobular carcinoma with pathological grade III, stage pT2mN0M0, and stage IIA with positive estrogen (60%) and progesterone receptor (60%) yet negative HER-2/neu status. The patient was planned to be given adjuvant chemotherapy, surgery, chemotherapy, and hormonal therapy.

Figure 3. The chronological axis of the patient’s medical history

Figure 4. Breast ultrasound (Asterisk showing irregular solid lesions with microcalcifications)
The patient started the chemotherapy on July 14, 2014, with cyclophosphamide, epirubicin, and a 5-fluorouracil (CEF) regimen, every 21 days for up to six cycles. The last cycle was on October 31, 2014. The patient started to receive tamoxifen 20 mg once daily on April 30, 2015. Then, the patient stopped taking tamoxifen for embryo transfer preparation on May 27, 2017.

On October 7, 2017, she started the treatment with estradiol valerate 2 mg for 16 days to prepare the endometrium before embryo transfer. After 16 days, ultrasound and estradiol were measured, and the treatment continued with estradiol valerate 2 mg and progesterone 400 mg. Four frozen embryos were thawed the day before embryo transfer and cultured in a CO2 incubator. Embryo transfer was performed on October 25, 2017, and three (grade-A embryos) were transferred. On November 6, 2017, the biochemical pregnancy test result was positive (human chorionic gonadotropin was 514 mIU/mL). On November 25, 2017, the patient underwent the first gestational ultrasound showing a gestational sac, and a heartbeat was positive. The patient was then referred to the obstetricians, and antenatal care showed normal pregnancy.

Pregnancy was terminated on July 1, 2018, by Caesarean section without any complications. A healthy boy was born with the normal term, birth weight, and length. The post-partum phase was normal, and breastfeeding for 2 months. Three months after giving birth, the patient started to take tamoxifen. The last follow-up was on April 1, 2022, finding that she had two healthy children and no complaints and evidence of disease. The second pregnancy was unplanned, and the patient was still breastfeeding the second child on the last follow-up. The chronology overview of this case is shown in Figure 6.

Then, the patient underwent an IVF procedure a month before starting the chemotherapy. The patient started controlled ovarian stimulation on June 16, 2014, with 150 IU r-FSH for nine days. The r-FSH dose was adjusted every two until four days, depending on the ultrasound and estradiol results. On June 26, 2014, eight follicles developed on the left ovary with the largest diameter were 22 mm, and ten follicles developed on the right ovary with the largest diameter of 25 mm (Figure 5). Ovulation induction was done with r-hCG on the same day. Oocyte retrieval was performed on June 28, 2014, with the results of 12 oocytes retrieved. In addition, there were two oocytes without polar bodies and ten oocytes with polar bodies. The oocytes with polar bodies were fertilized by ICSI on the same day. On June 29, 2014, nine oocytes were fertilized; nine embryos were developed in the next three days (grade A embryos). On July 2, 2014, all nine embryos were chosen to be frozen with the vitrification technique.

**Figure 5.** Transvaginal ultrasound showing follicles in the right and left ovary

**Figure 6.** The chronological axis of the patient’s medical history
DISCUSSION

Generally, chemotherapy induces toxicity to the ovaries by destroying primordial oocytes, granulosa cells, and ovarian stroma, leading to infertility. Regardless of the mechanism of action, all chemotherapy can damage the developing follicle by interfering with the development of granulosa cells, which in turn causes amenorrhea and can reflect permanent ovarian dysfunction [4].

Alkylating agents, such as cyclophosphamide, are also highly toxic to the ovaries [5,6]. Cyclophosphamide administration will impact primordial follicle reserves, and a decrease in follicular density of more than 90% has been reported to occur within 48 hours of the administration of cyclophosphamide [7]. In this case report, the patients were given CAF and CEF regimens. The CEF and CAF regimens were found to have a 40-60% risk of permanent amenorrhea in patients aged 30-39 years and >80% in those over 40 years [8].

Age at BC diagnosis also plays a role in determining the magnitude of the risk of infertility related to chemotherapy. As women grow older, the risk of infertility also gets higher. By 37 years of age, more than 95% of oocytes present at birth have undergone apoptosis. At 40 years, the fertility rate is 50% compared to a woman aged 30 years [9]. In addition, premenopausal BC patients are mostly more than 35 years old, so it is physiologically susceptible to infertility. In this case, the first case was considered more susceptible than the second one since she was older.

Consequently, patients of reproductive age diagnosed with BC should be informed about the risk of infertility and options for future fertility preservation before the start of any gonadotoxic therapy [10]. An oncologist’s early referral is also required if the patient’s condition allows fertility preservation [8,11]. Several fertility preservation methods can be done, including cryopreservation of oocytes, ovarian tissue, and embryos [12,13]. In this case, the patients were given counseling and referral regarding IVF and continued with embryo cryopreservation as the most effective and safe alternative to overcome chemotherapy-related infertility.

In our reported cases, IVF procedures were performed before starting chemotherapy. The IVF procedure usually begins with ovarian stimulation for about 10-14 days with gonadotropins to achieve multifollicular development [14]. In our report, the first reported patient was given r-FSH in 11 days, while the second reported it in nine days. Ovarian stimulation in a conventional IVF cycle almost always results in a 10 to 15-fold greater estradiol level than in a natural cycle [2]. Regarding the fact that increased estradiol levels may potentially be risky in hormone-dependent breast cancer patients (they could stimulate breast tissue growth again), controlled ovarian stimulation protocols have been recently developed. In our cases, the patients also underwent controlled ovarian stimulation. The r-FSH dose was adjusted every two to four days depending on the ultrasound and the results of estradiol levels, preventing spikes in high estradiol and ovarian hyperstimulation.

Moreover, in our cases, the vitrification method was applied for embryo cryopreservation. Cryopreservation of embryos is the gold standard procedure and has been used extensively for many years. Currently, the pregnancy rate following the transfer of frozen-thawed embryos is comparable to that following the transfer of fresh embryos [15]. Ovarian stimulation, mature oocyte retrieval, and IVF using sperm are the steps in embryo cryopreservation. There are two techniques for freezing embryos: vitrification and gradual freezing. In terms of pregnancy and live birth rates, several studies suggested that the embryo vitrification and thawing approach is preferable to gradual freezing and thawing. Embryo freezing has various benefits over oocyte cryopreservation, including lowering the risk of multiple pregnancies by limiting the number of embryos transferred and raising cumulative pregnancy rates [16].

Embryo transfer can be carried out after chemotherapy. It is advised that the postponement of pregnancy should be individualized based on treatment needs and prognosis. BC patients should wait six months to two years after completing the cancer treatment before attempting to conceive, as most cancer recurrences occur during this period, and both chemotherapy agents and hormonal therapy have teratogenic risks [11,12]. In addition, women who conceived more than or equal to one year after starting chemotherapy had higher risks of preterm birth more than or equal to one year after beginning chemotherapy without radiation or more than or equal to two years after chemotherapy with radiation [17]. However, the postponement of pregnancy should be individualized based on treatment received [18]. In the first case, the embryo transfer was performed a year after the last chemotherapy administration, and three years after the last chemotherapy in the second case. Both resulted in term labor.

Furthermore, hormonal therapy, such as tamoxifen, has a teratogenic effect on pregnancy. The half-life of tamoxifen and its metabolites is quite long; therefore, tamoxifen is suggested to be stopped two-three months before pregnancy as hormonal therapy has a teratogenic effect and suppresses prolactin release [19,20]. After that, it is recommended to complete hormonal therapy after pregnancy [17]. In this case report, the patients stopped taking tamoxifen one and five months before implantation.

Several favorable factors also support pregnancy success in these cases, including triple-positive BC profile (positive hormone receptor and positive Her-2 status) in the first patient, making her more sensitive to hormone treatment and chemotherapy; absence of metastases in both patients; BC management facilities...
and fertility preservation protocols. In both cases, the pregnancies went well.

Generally, studies did not show a worse outcome for previously diagnosed and treated women with BC who desired to become pregnant afterward [21,22]. There was also no poorer disease-free survival in premenopausal women with estrogen receptor-positive BC that became pregnant within five years of diagnosis [23]. In addition, the study showed no higher proportion of metastases in women who gave birth after BC than those not pregnant [24]. However, careful examination of the breast and a multidisciplinary approach are still recommended, as increased estrogen during pregnancy is suspected to accelerate cancer growth, leading to recurrence [20].

In addition, comparative studies between IVF and embryo cryopreservation in cancer patients to those without cancer did differ significantly in the number of harvested oocytes, fertilization rate, and live birth rate. Nevertheless, there were few good-quality embryos in patients with cancer [16]. Women who had received chemotherapy showed no increased risk of miscarriage or stillbirth [25]. The infant born was also not at increased risk for low birth weight and congenital malformations compared to the general population [20,26], explaining healthy and normal-term babies delivered by those patients.

Breastfeeding after BC is also allowed as it is not contraindicated for women with no residual tumor [27]. There are no epidemiological data on the impact of breastfeeding on the risk of contralateral BC or the risk of recurrence in the ipsilateral breast [26]. Besides, there is no evidence that breastmilk from mothers previously treated for BC increases the risk of disease in children. However, BC treatment, especially surgery and radiotherapy, may interfere with breastfeeding due to reduced milk production [20].

CONCLUSIONS

Pre-chemotherapy IVF continued with embryo cryopreservation is a safe and effective potential alternative to overcome infertility in women of reproductive age who are receiving chemotherapy. A healthy pregnancy can even occur after chemotherapy with no consequences for the fetus or later for the child.

DECLARATIONS

Competing interest
The authors declare no competing interest in this study

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REFERENCES