Fertility Preservation in Female Cancer Patients

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Abstract

Modern therapies for cancer such as surgery, chemotherapy, and irradiation have greatly improved survival rates. However, the treatment that serves to prolong life often comes with long-term side effects. In women of reproductive age and younger, one of the side effect is premature ovarian failure leading to infertility. At present, several options are available for the preservation of fertility. These include ovarian transposition, embryo, oocyte, and ovarian tissue cryopreservation. Hence, reproductive-aged women with cancer should be counseled about fertility preservation before initiating therapy. The choice of the most suitable strategy for preserving fertility depends on many factors such as the cancer type, patient age, type, dose, and length of chemotherapy, or the dose of radiation given and the area being irradiated. This review discusses the available methods to preserve female fertility and/or ovarian function in young cancer survivors, and also proposes an algorithm guideline for an individualized approach to fertility preservation.

Keywords: assisted reproductive technology, fertility preservation, cancer patient

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Introduction

Advances in treatment of malignancy can significantly improve survival rates. To date, more than 90% of young women affected by cancer can be cured. However, a significant proportion of these survivors will be rendered sub-fertile or sterile as a result of aggressive chemotherapy or radiotherapy. Oocytes are very susceptible to damage from chemotherapeutic agents and radiation. The side effects of chemotherapy depend on the dose, drug type, and age of the patient. Women who are treated with alkylating agents, such as cyclophosphamide, busulfan, melphalan, chlorambucil are considered to be at high risk for gonadal dysfunction. With regard to radiotherapy, there is evidence that a dose of $< 2$ Gy can damage 50% of the ovarian reserve, while a dose of 5–20 Gy would result in a complete destruction of the ovarian tissue. Given that a number of patients desire to have children after their treatments, several methods of preserving fertility have been proposed to ensure a chance for a future pregnancy.

The aims of this article are to review the fertility preservation methods, options, patient candidates, method selection and success rates in term of pregnancy in young cancer patients.

Fertility preservation options

Fertility preservation treatment should always be considered for prepubertal girls and young women who are going to undergo cancer treatment. These patients must receive clear information about the possible side effects of the therapy, including fertility-related topics.
The options for fertility preservation depend on the patient age, partner status, cancer type, type, dose and length of chemotherapy or the dose of radiation given and the area being irradiated. These methods include ovarian transposition, cryopreservation of embryos and oocytes, and more recently ovarian tissue cryopreservation. Embryo and oocyte cryopreservation techniques have been extensively studied over the past three decades; however, there are still some limitations in clinical application. In order to freeze embryos, either sperm from the woman’s male partner or sperm from a sperm donor is needed. On the other hand, oocyte cryopreservation carries a high risk of aneuploidy and its success rate is still limited. Moreover, both methods require a delay in cancer treatment by several weeks to carry out ovarian stimulation and oocyte collection, which is not always feasible when a patient requires urgent commencement of cancer treatment, or has contraindications for hormonal treatment, depending on the cancer type. One modern innovation is the auto-transplantation of frozen–thawed ovarian tissues or the whole ovary with its pedicle which will be re-implanted to the patient after they have been cured of their cancer. An algorithm for fertility preservation according to risks of ovarian involvement and methods of cancer treatment is shown in Fig. 1.

**Ovarian transposition**

Pelvic radiation is often indicated in some women with genitourinary, Hodgkin’s disease, or colorectal cancer. Radiation may be administered locally or to a larger area, depending on the site and the extent of the disease. Although highly effective in cancer treatment, pelvic

![Diagram of Fertility Preservation Methods](image-url)

**Fig. 1** Schematic overview of fertility-preserving methods for prepubertal girls and young women (modified from Oktay and Sonmez).
radiation may result in the loss of ovarian function. Ovarian transposition is a method to remove the ovary from the field of radiation.\(^a\) This method has been used for more than 3 decades to preserve ovarian function in lymphoma patients who receive pelvic or para-aortic lymph node irradiation. Its success rates of fertility preservation range from 16% to 90%, depending on several factors such as vascular compromise, scatter radiation, radiation dose, and age of the patient.\(^b\) This technique can be performed by either laparotomy or laparoscopy, depending on surgical skill and operative experience of the gynecologist.\(^c\)

For the patient who desires to have a child after ovarian transposition, in vitro fertilization (IVF) with a surrogate mother may be needed. William et al\(^d\) reported the outcomes of laparoscopic ovarian transposition to preserve ovarian function prior to pelvic radiation in 12 patients who wished to have children. At their follow-up visits, five patients (41.7%) had evidence of ovarian function and four of them (80%) achieved pregnancies.

**Embryo cryopreservation**

IVF to produce embryos for cryopreservation is an established method for fertility preservation. It is routinely used in assisted reproductive technology (ART) clinic. With recent improvements in the freezing methods, the pregnancy rates from frozen–thawed embryos are similar to those from fresh embryos.\(^e\)

Although this method is an efficient technique, there are several significant drawbacks. First of all, the selected patient must be of pubertal age and has a partner or otherwise consents to receive donated sperm.\(^f\) Secondly, embryo cryopreservation requires ovarian stimulation at least two weeks and is therefore contraindicated if the initial treatment of cancer can not be delayed. Besides, the ovarian stimulation may be harmful to the patients with estrogen sensitive tumors, such as breast cancer, as these cancers can potentially be affected and enhanced by increased hormone levels administered during the stimulation phase prior to oocyte collection.

Results from the 10\(^{th}\) European IVF–monitoring report\(^g\) in the year 2006 showed a 12.7% live birth rate with frozen–embryo transfer, compared with a 21.5% rate with IVF aspiration and 18.4% rate with intracytoplasmic sperm injection (ICSI) aspiration. According to the Centers for Disease Control and Prevention (2008),\(^h\) a live birth rate of non–donor cycles from frozen embryos was 33.2%. These rates were somewhat higher than those being reported in the European countries.

**Oocyte cryopreservation**

Oocyte cryopreservation is an option for fertility preservation. It is suitable for the patients who do not have a current partner or do not wish to use donated sperm. Oocytes can be preserved either as mature or as immature oocytes.

**Mature oocyte cryopreservation**

Mature oocytes or metaphase II (M II) oocytes can be frozen and stored as an option for women without a partner who might wish to have children at a later time. However, oocytes are more difficult to cryopreserve than cleavage–stage embryos or zygotes. Historically, this method has had lower success rates than standard IVF.\(^i\)

There are two primarily available options for mature oocyte cryopreservation, including slow–freezing and vitrification. The majority of oocyte cryopreservations have used the slow–freezing method, as this method has been used longer than the more recent vitrification.\(^j\) However, results of this procedure have been disappointing with low survival, fertilization and pregnancy rates after IVF of thawed oocytes.\(^k\) The pregnancy rates in most studies were less than 2% per thawed oocyte.\(^l\)

The M II oocyte is a large and fragile cell which
is susceptible to a wider range of cell injuries. During the freezing–thawing process, the damage of subcellular structures such as meiotic spindle, mitochondria, or cortical granules could account for the low survival rates.\textsuperscript{17} Damage to the meiotic spindle apparatus, along with the chromosomes align at metaphase, is detrimental to oocyte viability. Ice formation during the freezing–thawing process results in the depolymerization of the meiotic spindle, and this disruption causes a risk of aneuploidy.\textsuperscript{6} After the freezing–thawing process, the zona pellucida will become hard. Although a hardened zona becomes an obstacle for sperm to penetrate the zona, this can be bypassed with intracytoplasmic sperm injection (ICSI).\textsuperscript{18} However, even after using ICSI to fertilize a frozen–thawed oocyte, the reports of pregnancy rates remained low.\textsuperscript{7}

Recently, a new vitrification method has been introduced in ART, and the re–fertilization success rates have been constantly improving since then.\textsuperscript{19} A meta–analysis\textsuperscript{13} showed that live–birth rates for thawed oocytes with a slow–freezing method were significantly lower than those for fresh oocytes using the same freezing protocol (15.4\% vs 38.4\%, \emph{p}–value < 0.0001). On the other hand, there was no significant differences in live birth rates between thawed and fresh oocytes using the same vitrification technique (46.6\% vs 43.9\%, \emph{p}–value = 0.09). Although the technique of oocyte cryopreservation seems to be improved, it is still considered experimental. More studies are needed to provide valuable and safety data of this technique in clinical practice.

\textbf{Cryopreservation of immature follicles with in vitro maturation (IVM)}

Unlike freezing mature oocytes, this process of oocyte freezing involves retrieval of immature oocytes at the germinal vesicle (GV) stage with subsequent in vitro maturation prior to fertilization and consequent embryo transfer.\textsuperscript{20} The advantage of using immature oocytes as opposed to mature oocytes is that the ovarian stimulation phase is not mandatory and cancer patients do not need to postpone treatment. Moreover, oocytes at the GV stage survive the freezing process better than the larger, more fragile mature oocytes. The immature oocytes, which have not initiated the second phase of metaphase, have the advantage in that the meiotic spindles have not yet developed and the nuclear membrane has still enveloped around the chromat. In this case, the cytogenetic anomalies are less likely to occur during the freezing–thawing process at this stage in their development.\textsuperscript{21} An additional advantage of using immature oocytes over the use of mature oocytes is that it is less expensive.

Lately, live births following vitrification of in vitro matured oocytes have been reported from several ART centers.\textsuperscript{22–24} However, it is still difficult to achieve high success rates of in vitro maturation (IVM) and this treatment still results in low birth rates.\textsuperscript{22–24} The future improvements of IVM culture conditions and follicle regeneration rates in frozen–thawed tissue may yield better survival outcomes, and allow this method to become a valid option for fertility preservation in clinical practice.

\textbf{Cryopreservation of ovarian tissue}

Cryopreservation of ovarian tissue is an available option for prepubertal girls and women who cannot delay commencement of chemotherapy or who have contraindications for the hormonal drugs used for ovarian stimulation. This procedure can preserve both fertility and ovarian function after autotransplantation of ovarian tissues. Frozen–thawed ovarian tissue can be autografted to the origin (orthotopic) site\textsuperscript{2} or to alternative (heterotopic) sites.\textsuperscript{25} Moreover thawed ovarian tissue can be grafted to severe combined immunodeficiency mice called xenograft to avoid reintroduce malignant cells to the patient.\textsuperscript{26}

There are several ways to transplant ovarian tissue: A. According to the relationship between the donor and recipient of the tissue:

- Autograft: where the graft is taken from and re–implanted back into the same patient. This type of
graft eliminates the risk of tissue rejection; however, it may create the potential risk of transferring metastatic cells in the graft tissue in cancer patients.\textsuperscript{27}

- Isograft: where the recipient of the graft is the same genotype as the donor, e.g. an identical twin. This technique also presents little risk of immunological complications.

- Allograft: where the tissue is taken from one individual and grafted into a genetically dissimilar individual of the same species, e.g. between unrelated people. This technique has a high risk of graft rejection.

- Xenograft: where tissue is taken from one individual and grafted into another individual of a different species. This requires the recipient individual to be immunologically compromised.

B. According to the site of implantation:

- Orthotopic: where the graft is replaced into its physically normal position.

- Heterotopic: where the tissue is grafted to an atypical site.

There are two methods for preservation of ovarian tissue, including preservation of cortical strips and preservation of a whole ovary with its pedicle.

- Cryopreservation of cortical strips

With regard to cryopreservation techniques, ovarian cortical strips are easier to freeze and thaw than the whole ovary. By using frozen–thawed cortical strips, the achievement of pregnancies and the restoration of hormone production have been reported in sheep.\textsuperscript{28} The first live birth has also been reported in humans in 2004.\textsuperscript{29} However, the report of human involved case in which the whole ovary was not removed, and at that time the authors could not guarantee that all ovarian follicles will be damaged after the treatment. Moreover, the patient had at least one ovulatory cycle following confirmation of ovarian failure. This has led to arguments from some authors that the origin of pregnancy may be derived from the native ovary rather than the transplanted frozen–thawed cortical strips.\textsuperscript{30}

A major problem of using frozen–thawed cortical strips is that the restoration of ovarian function is temporary because the grafts have only a limited life span.\textsuperscript{22,28} With the classical slow freezing technique, the total follicle loss following grafting of frozen–thawed cortical strips is as high as 50 to 65\%.\textsuperscript{35,31} Likewise, Aubard et al\textsuperscript{35} reported more than 90\% loss of oocytes by using this technique. Current evidence suggests that most follicles are lost due to ischemic reperfusion injury rather than through the actual freezing–thawing process.\textsuperscript{28} Baird et al\textsuperscript{35} also demonstrated that 65\% of primordial follicles were lost during transplantation, whereas only 7\% of primordial follicles were lost during freezing and thawing. Fig. 2 shows the recoveries of ovarian follicles after transplantation of fresh versus frozen–thawed ovarian tissues of ewes. It is apparent that the number of follicles in frozen–thawed ovarian tissue was significantly lower than that of fresh ovarian tissue. The main cause of follicle loss in the autotransplantation of ovarian tissue is hypoxia, since the grafted tissues did not have their own vascular supply and have to depend on neovascularization, which takes up to a week following the grafting procedure.\textsuperscript{33}

Because of the very high rates of ovarian follicle loss after transplantation, the functional life span of a cryopreserved ovarian tissue is limited but likely to be up to about 3 years.\textsuperscript{34,35} To extend the duration of ovarian function, multiple transplantations of cryopreserved ovarian tissues will be required.\textsuperscript{34} Regarding pregnancy outcomes, so far 13 healthy babies have been born following orthotopic transplantation of cryopreserved ovarian tissue. Most patients conceived spontaneously.\textsuperscript{35} For heterotopic transplantation, no clinical pregnancies have been reported from retrieved oocytes.\textsuperscript{35,36}

Several techniques have been developed in an attempt to improve follicle survival through the freezing–thawing processes, including techniques to increase the blood supply to the cortical tissues after transplantation:

**Cryopreservation of a whole ovary with its pedicle**

One of the most promising techniques for im-
proving the survival of transplanted oocytes is whole ovarian cryopreservation with vascular reanastomosis.\textsuperscript{37–39}

This process is intended to increase the number of viable follicles and to provide the possibility of long-term restoration of ovarian function. Several studies have demonstrated that ovarian tissues can survive and produce ovarian hormones after autotransplantation of whole frozen–thawed ovary with vascular reanastomosis.\textsuperscript{37–39}

This technique of whole ovarian cryopreservation seems to be more theoretically suitable than cryopreservation of ovarian cortical strips, as preserving the whole ovary enables the patient to avoid ischemia after a frozen–thawed transplantation. This method should result in more surviving primordial follicles and prolong the full ovarian function.\textsuperscript{37} However, the drawback of whole frozen ovary transplantation is the difficulty of adequate diffusion of cryoprotective agents, especially in a human ovary which is larger than ovaries of other mammalian species.\textsuperscript{40} Practically, a technique to cryopreserve the whole ovary in a large species such as sheep or humans is different from that of smaller species such as rats and mice. In smaller species, the method can be achieved without widespread perfusion of a cryoprotectant, just using simple diffusion, whereas the larger ovaries in larger species are more fibrous, and thus require more attention to the perfusion. Basically, to preserve the whole ovary, we need to cannulate ovarian artery to perfuse cryoprotective agents into the tissue. This process may cause endothelial damage leading to an increase risk of thrombotic events after transplantation.\textsuperscript{37,41}

The outcomes of transplantation of whole frozen–thawed ovaries with vascular pedicles in sheep have been reported.\textsuperscript{37–39} Bedaiwy et al\textsuperscript{37} demonstrated the success of transplantation of whole frozen–thaw ovaries. However, the vascular pedicles were patent in only 27\% of the grafts. The rest of the grafts were lost due to the thrombosis of reanastomosed vascular pedicles. A later study by Arav et al\textsuperscript{40} reported that 5 out of 8 ovaries were successfully autotransplanted in terms of immediate vascular patency. In the long term, only two ewes

\textbf{Fig. 2} Recoveries of the follicles in cortical patches after transplantation of fresh (a) versus frozen–thawed (b) ovarian tissues (Hematoxylin & Eosin staining).
Table 1  Summary of patient candidate, advantages, disadvantages and pregnancy rates among options for fertility preservation

<table>
<thead>
<tr>
<th>Options</th>
<th>Patient candidate</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Pregnancy rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian transposition</td>
<td>receiving pelvic radiation</td>
<td>established method</td>
<td>need IVF and surrogate mother if patients want to have a child</td>
<td>16–90%&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Embryo cryopreservation</td>
<td>having a partner or accepting donor sperm</td>
<td>established method</td>
<td>– delay treatment 2–4 wks</td>
<td>13–33%&lt;sup&gt;10,13&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oocyte cryopreservation</td>
<td>do not have a partner or prepubertal girl</td>
<td>– some reported pregnancy rate almost similar to embryo cryopreservation</td>
<td>– delay treatment 2–4 wks</td>
<td>15–47%&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Mature oocyte</td>
<td></td>
<td>– not delay treatment</td>
<td>– contraindicate for estrogen sensitive tumor</td>
<td></td>
</tr>
<tr>
<td>- Immature oocyte</td>
<td></td>
<td>– less expensive</td>
<td>– experimental procedure</td>
<td>no data</td>
</tr>
<tr>
<td>Ovarian tissue cryopreservation</td>
<td>do not have a partner or prepubertal girl</td>
<td>– preserving ovarian and fertility function</td>
<td>– risk of reintroduce cancerous cells</td>
<td>available</td>
</tr>
<tr>
<td>- Cortical strips</td>
<td></td>
<td>– preserving a great number of oocytes</td>
<td>– experimental procedure</td>
<td></td>
</tr>
<tr>
<td>- Whole ovary with pedicle</td>
<td></td>
<td></td>
<td></td>
<td>at least 13 live births reported&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

maintained cyclic progesterone production over 6 years post–transplantation.<sup>42</sup> Ihmof et al<sup>29</sup> reported a live birth following autotransplantation of intact frozen–thawed sheep ovaries with microanastomosis of the ovarian pedicle. In humans, only few studies of whole ovarian cryopreservation have been reported. Martinez–Madrid et al<sup>40</sup> demonstrated a survival rate of follicles following thawing of 75.1%. To date, however, there seems to be no advantage of a whole ovary transplantation by vascular anastomosis compared with ovarian tissue transplantation, because of the still-existing poor follicular survival and higher surgical risks.<sup>43</sup>

**Risk of reimplantation**

One of the main concerns regarding the autotransplantation of ovarian tissue from cancer patients is the
risk of re-introducing cancerous cells. There has been clear evidence of transmission of cancer cells via grafts of ovarian tissue, especially blood-borne malignancies. Fortunately, most of the cancers of reproductive age women do not metastasize to ovaries. Cancers with high risk of ovarian involvement include leukemia, neuroblastoma, Burkitt lymphoma and some advanced stage solid tumors such as breast and colon cancers. Therefore, these cancer patients may be better advised to have other alternative methods of fertility preservation. However if there is no other option, ovarian tissue can be biopsied and assessed for the presence of malignant cells or minimal residual disease using tumor specific chromosome markers, histochemical markers or polymerase chain reaction (PCR) methods. Although this still carries a risk, cancer-free biopsied can then be selected for cryopreservation and grafting. Some cancers, such as Hodgkin’s lymphoma, Wilm’s tumor, Ewing’s sarcoma or breast cancer stage I–III, are unlikely to be transmitted in an ovarian graft and may therefore be safe to offer this technique in these cases.

Conclusion

A significant number of young cancer patients surviving from chemotherapy and/or radiotherapy will face the problem of premature ovarian failure. To preserve their fertility for a future pregnancy, several methods including ovarian transposition, embryo, oocyte and ovarian tissue cryopreservation have been utilized. Ovarian transposition and embryo cryopreservation are well-established methods whereas oocyte and ovarian tissue cryopreservation are still considered as experimental methods. Patient candidate, advantages and disadvantages among these options for fertility preservation were summarized in Table 1.

References


