

Fertility preservation and pregnancy outcome after malignancy

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Purpose of review

The overall survival and cure rates of patients with childhood and adult malignancies have improved dramatically, but cancer treatment can be associated with diminished reproductive potential. However, research on the preservation of fertility in these patients has given patients new options. This article discusses the mechanisms of reproductive failure after cancer therapy and the currently available fertility preservation strategies.

Recent findings

Ovarian transposition is still a viable option if radiotherapy is to be used alone. Modifications in assisted reproductive technology that decrease peak estradiol levels are ideal for breast cancer survivors. Embryo freezing technology offers excellent pregnancy rates. Oocyte freezing is available for women without a partner, but there is more limited experience with this technique. Understanding the concepts of graft function after the autotransplantation of frozen–thawed ovarian tissue has resulted in great strides in the technical requirements for success.

Summary

Gonadotropin-releasing hormone analogues are the only available medical protection means for gonadotoxic chemotherapy. Assisted reproductive technology offers excellent results, but the protocols require a delay in implementing chemotherapy. Despite recent reports of embryo development after the transplantation of cryopreserved–thawed ovarian tissue, clinical experience is limited and the technique remains experimental.

Keywords

chemotherapy, fertility preservation, ovarian failure, radiotherapy

Introduction

The treatment of childhood and adult malignancies carries a substantial risk of compromise of reproductive potential. Many patients are expected to survive these cancers and lead normal lives. Consequently, fertility preservation has become more important. Besides the expected difficulties with fertility, pregnancy-associated disorders such as early pregnancy loss, premature labor and low birthweight have been described after cancer treatment [1]. In this article we will review recent literature on the pathophysiology of chemotherapy/radiotherapy-induced gonadal toxicity and recent data on the outcome of techniques used for fertility preservation. Although the focus of this review is on the cancer patient, the comments also apply to other disorders such as autoimmune disease, which often occurs in reproductive age women who are treated with chemotherapy.

Chemotherapy-induced ovarian failure

Premature ovarian failure (POF) is a well-known consequence of exposure of the female gonad to chemotherapeutic drugs. A wide variety of malignant and non-malignant conditions during the reproductive age are treated with gonadotoxic chemotherapy. Poirot *et al.* [2] reported on a group of women under 16 years of age who requested preservation of fertility with ovarian tissue cryopreservation. The tumors in these patients are listed in Table 1.

Breast cancer is the commonest malignancy in women during the reproductive age that often requires immediate fertility intervention [3••]. Fifteen per cent of all breast cancer cases are estimated to occur at less than 40 years [4]. Cervical cancer is the other common malignancy seen in reproductive age women that may require fertility-preserving intervention. It is estimated that 50% of the 13 000 new cervical cancer cases that were diagnosed in the United States are under the age of 35 years [5•].

Chemotherapy-induced gonadotoxicity is almost always irreversible. Histological sections of the ovary after treatment with cytotoxic drugs known to cause ovarian failure show a spectrum of changes ranging from decreased numbers of follicles to absent follicles to fibrosis. The exact incidence of POF after chemotherapy is difficult to establish because many factors contribute to ovarian failure. The most important parameters are the age of the patient, the drug class, and the cumulative dose of the drug. The risk of gonadal damage increases as the age of

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Abbreviations

ART assisted reproductive technology
FSH follicle-stimulating hormone
GnRH gonadotropin-releasing hormone
IVF in-vitro fertilization
POF premature ovarian failure

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Table 1. Most frequent cancer types in patients requesting fertility preservation

Childhood cancers
Ewing's sarcoma
Leukemias
Lymphomas
Neuroblastomas
Wilm's tumor
Adult tumors
Breast cancer
Cancer of the cervix
Colorectal carcinoma
Lymphomas
Sarcomas

the woman increases and is most likely caused by the presence of a lower number of remaining oocytes compared with the younger patient. Cytotoxic chemotherapeutic agents are not equally gonadotoxic (Table 2). Chemotherapy that includes alkylating agents is particularly gonadotoxic. These drugs may impair follicular maturation or deplete primordial follicles. Temporary amenorrhea will result when maturing follicles are destroyed by cytotoxic drugs, whereas permanent amenorrhea or POF is seen when all primordial follicles are destroyed. A fascinating observation was recently reported in a paper in *Nature* [6**]. The dogma of ovarian physiology is that germline stem cells do not occur in the postnatal ovary. In other words, there are no cells available to generate new oocytes. If primordial cells are depleted (menopause) or destroyed through therapy, no new oocytes are generated. That study showed the presence of germline stem cells in the adult ovary and therefore the potential for the regeneration of new oocytes if depleted by chemotherapy. Methods that specifically protect these cells may lead to new therapies to replenish destroyed follicles.

Radiotherapy-induced gonadal damage

Several cancers afflicting young premenopausal women can be cured with radiation therapy. The degree and

Table 2. Gonadotoxicity of different chemotherapeutic agents

Low risk
Methotrexate
5-Fluorouracil
Actinomycin D
Bleomycin
Vincristine
6-Mercaptopurine
Intermediate risk
Cisplatin
Adriamycin
High risk
Alkylating agents
Cyclophosphamide
Busulfan
Melphalan
Nitrogen mustard

Source: Sonmezer and Oktay [16*], reproduced with permission.

persistence of ovarian damage is related to the patient's age, the total dose, and the number of episodes needed to deliver the dose. Radiation is more toxic when given in a single dose compared with fractionated doses [7]. Two studies indicated that the breakpoint for radiation-induced ovarian failure is approximately 300 cGy to the ovaries. Only 11–13% experienced ovarian failure below 300 cGy versus 60–63% above that threshold value [8]. The radiation doses to the ovaries with standard pelvic radiation therapy will uniformly induce ovarian failure. The addition of chemotherapy increases the risk of POF [9,10].

The ovarian follicles are remarkably vulnerable to DNA damage from ionizing radiation, resulting in a significant reduction in the ovarian follicle pool. Oocytes show a rapid onset of pyknosis, chromosome condensation, disruption of the nuclear envelope and cytoplasmic vacuolization. Serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone increase progressively within 4–8 weeks after radiation exposure along with a decline in serum estradiol levels. A dose-dependent reduction in the primordial follicle pool was noted upon exposing the ovary to radiotherapy [11]. It is estimated that less than 2 Gy is enough to destroy 50% of the oocyte population ($LDL_{50} < 2$ Gy) [12].

Fertility preservation strategies

A wide array of options have been tested as fertility preserving strategies. However, none of them was tested in a prospective randomized controlled trial. Many of the available techniques are promising but are still experimental techniques.

Pharmacological protection

After noting that the premenarchal ovary appears to be less sensitive to cytotoxic drugs [13,14], investigators attempted to mimic this state by using gonadotropin-releasing hormone (GnRH) agonists. Previous animal studies in different species had shown that GnRH agonists could protect the ovary against cyclophosphamide-induced damage. Blumenfeld [15*] reported several studies on the potential protective effects of GnRH agonists against the deleterious effects of chemotherapeutic agents. All the reports demonstrated a diminished frequency of POF in patients who received a GnRH agonist before starting chemotherapy. However, the retrospective nature of the control group makes the observations less robust. The length of follow-up was not comparable between the two groups, with the GnRH agonist group having a shorter follow-up. From such studies, it cannot be determined whether the lower incidence of ovarian failure was caused by GnRH agonist treatment or the shorter follow-up.

Some authors have questioned the value of GnRH gonadal suppression in women in preserving ovarian function

against chemotherapy [16[•]]. Primordial follicles initiate follicle growth through an unknown mechanism, which is gonadotropin independent. This was exemplified in a recent study of a patient with an FSH receptor mutation that presented with POF but with normal follicle development to the small antral stage [17^{••}]. There is some controversy regarding the existence of GnRH receptors on the human ovary, whereas GnRH receptors have clearly been detected in the rat ovary. The response may thus not be similar across species. If the sole mechanism of gonad protection with GnRH agonists were through the suppression of gonadotropins, especially FSH, then treatment would not be expected to protect the primordial follicle population that represents the ovarian reserve.

Some prepubertal children receiving gonadotoxic chemotherapy may eventually have POF [18]. Meiwor *et al.* [19] proposed that as younger patients have a larger ovarian reserve a decreased frequency of immediate amenorrhea does not mean that the gonads are unaffected by the chemotherapy, but simply that they have a sufficient number of oocytes not to demonstrate immediate ovarian failure. This controversy on the effectiveness of GnRH agonists will only be resolved with a prospective randomized clinical trial. Suppressive therapy with a variety of oral steroids such as oral contraceptives or progestins has not been shown to be effective in preventing damage from chemotherapy or radiation therapy.

Ovarian transposition

For patients subjected to gonadotoxic radiotherapy, ovarian function could be maintained by transposing the ovaries out of the field of irradiation. The ovarian dose after transposition is reduced to approximately 5–10% of that of in-situ ovaries [20].

Lateral ovarian transposition was typically performed by laparotomy, with division of the utero-ovarian ligament and tube and the ovaries were moved to the paracolic gutters so that they would lie 3 cm above the upper border of the field. Bidzinski *et al.* [21] confirmed that ovarian function was preserved if they were transposed at least 3 cm from the upper border of the field. Ovarian failure may result if the ovaries are not moved far enough out of the radiation field or if they migrate back to their original position. Currently, it has been recommended that ovarian transposition is performed laparoscopically just before initiating radiation therapy. Bisharah and Tulandi [22^{••}] reported a case of laparoscopic lateral ovarian transposition in a patient with rectal adenocarcinoma, whereby the utero-ovarian ligaments were divided but the ovaries remained attached to the distal fallopian tubes, potentially improving the chances for ovum pick-up. The patient achieved a spontaneous pregnancy.

An important advantage of laparoscopic ovarian transposition is that radiation therapy can be initiated immediately postoperatively preventing failure as a result of the ovaries migrating back. A further advantage of the laparoscopic approach, which offers rapid postoperative recovery, is the possibility of repeating the laparoscopy again if the postoperative assessment of the transposed ovaries shows that the radiation dose is still significant.

Several papers addressed concerns regarding pregnancy outcomes after pelvic irradiation. Swerdlow *et al.* [23] confirmed that there was no excess of stillbirths, low birthweight, congenital malformations, abnormal karyotypes, or cancer in the offspring of women treated for Hodgkin's disease. However, Fenig *et al.* [24] cited an increase in low birthweight and spontaneous abortions, especially if conception occurred less than a year after radiation exposure. They advised delaying pregnancy for a year after completing radiation therapy.

Assisted reproductive technology

The patient also has the option of preserving gamete function *ex vivo* with assisted reproductive technology (ART). In women without a partner, freezing mature or immature oocytes may be the only practical option. The main factor that may influence the outcome in oocyte cryopreservation is its structural complexity. A variety of cellular and subcellular structures of the human oocyte, such as the zona pellucida and meiotic spindle, appear to be very sensitive to cryopreservation damage.

Despite the early disappointing results, with low survival, fertilization, and pregnancy rates after the in-vitro fertilization (IVF) of thawed oocytes, recent studies have suggested increased success rates [25,26[•],27[•]]. Porcu and colleagues [28^{••}] recently instituted a programme of oocyte cryopreservation for oncology patients. Over a period of 4 years, 18 patients awaiting chemotherapy and radiotherapy for neoplastic disease were offered oocyte cryopreservation. Pregnancies occurred after fertilization and transfer of the cryopreserved oocytes. Pregnancies occurred even with oocytes that had been cryopreserved for years. Several techniques were introduced that have improved pregnancy rates with oocyte cryopreservation. First, rather than the traditional slow freezing protocols that are used for embryos, rapid freezing by vitrification was introduced and improved oocyte thaw survival [25,26[•],27[•]]. Second, the use of intracytoplasmic sperm injection has improved fertilization rates [28^{••}]. Vitrification protocols typically use ethylene glycol and dimethylsulphoxide as cryoprotectants. Yoon and colleagues [27[•]] reported survival rates of 68%, fertilization rates of 71%, and a pregnancy rate of 21% per embryo transfer. However, only seven babies were obtained from the cryopreservation of 474 oocytes. Although progress

has been made, the process requires improvement before this approach can replace embryo cryopreservation.

Embryo cryopreservation was introduced to maximize the conception chances from a single cycle. The human embryo is very resistant to damage from cryopreservation. The post-thaw survival rate of embryos ranges between 35 and 90%, and if multiple embryos were available for cryopreservation cumulative pregnancy rates can be more than 60% [29]. Delivery rates per embryo transfer utilizing cryopreserved embryos are reported by the Society for Assisted Reproductive Technology to be in the range of 18–20%. This is the option with the best outcome for the patient. This option may not be acceptable to prepubertal, adolescent girls, and women without a partner. However, if acceptable, long-term data are available about the outcome of children born from these procedures.

A potential problem with breast cancer patients is the extremely high estradiol levels achieved with IVF. Typically, there is an interval of 6 weeks between surgery and the initiation of chemotherapy for breast cancer. Rather than the standard protocol, a short flare protocol is used that usually requires less time to achieve follicle recruitment. Similarly, in patients with cancer of the cervix rather than the standard protocol, a short flare protocol is used that usually requires less time to achieve follicle recruitment [30]. However, there is some concern that in patients with breast cancer the high estrogen levels obtained in an IVF cycle may impact on long-term survival. For this reason, some centers have offered natural cycle (unstimulated) IVF. A single oocyte is usually aspirated. However, cancellation rates are high and the pregnancy rates are very low (7.2% per cycle and 15.8% per embryo transfer) [31^{••},32]. Conventional controlled ovarian hyperstimulation is associated with a significant increase in serum estrogen, which might affect the overall prognosis. Consequently, tamoxifen, a non-steroidal antiestrogen commonly used in breast cancer patients, was recently investigated for ovarian stimulation and IVF in breast cancer survivors [31^{••}]. Tamoxifen 40–60 mg was started on day 2 or 3 of the cycle and was given daily for 5–12 days. The control group consisted of patients who had an unstimulated IVF cycle. The tamoxifen group had a significantly higher numbers of mature oocytes, peak estradiol, and embryos (mean of 1.6 embryos versus 0.6 embryos) than the natural cycle group [31^{••}].

Recent work has focused on other drugs that try to maintain low estradiol levels during IVF cycles. Aromatase P450 aromatizes androgens to estrogens. Letrozole is a potent and highly selective third-generation inhibitor that competitively inhibits the activity of aromatase enzyme [33]. Letrozole significantly suppresses plasma estradiol, estrone and estrone sulphate levels. Letrozole

was shown to be more effective than tamoxifen in the treatment of advanced stage postmenopausal breast cancer [34^{••}].

Moreover, letrozole was introduced as a promising ovulation induction agent. Repeated clinical studies documented its clinical feasibility in ovulation induction. It could be used independently or in combination with FSH. It was also used for the treatment of poor responders [35^{••}]. Many groups are currently testing the feasibility of ovarian stimulation with aromatase inhibitors in breast and endometrial cancer patients. The patient is stimulated with gonadotropins and an aromatase inhibitor is simultaneously introduced to reduce serum estradiol levels. Oocyte development is unaffected. A luteinizing hormone-releasing hormone antagonist is also used to prevent a premature luteinizing hormone surge.

A recent study compared pre-cancer treatment ART and embryo cryopreservation with post-cancer treatment ART and immediate transfer. As expected, women undergoing IVF after chemotherapy had a poorer response to gonadotropins and produced fewer embryos and fewer pregnancies [36]. A recent case report exemplified this treatment approach of performing IVF before rather than after chemotherapy [37[•]]. Controlled ovarian stimulation, IVF, and embryo freezing were performed before fluorouracil-based chemotherapy in a 28-year-old woman who underwent subtotal colectomy for colorectal cancer. Three years later, when the clinical and hormonal analysis confirmed ovarian failure, two thawed embryos were transferred to the uterus. She gave birth at term to a 3200 g infant.

Cryopreservation and transplantation of ovarian tissue

Ovarian tissue cryopreservation and transplantation is an experimental procedure introduced to preserve fertility in women with threatened reproductive potential. Unlike a suspended single cell, tissue cryopreservation presents serious physical constraints related to heat and mass transfer and the potential formation of ice crystals, which is responsible for the freeze–thaw injury [38]. Consequently, better survival is expected from primordial follicles because of their smaller size and lack of follicular fluid.

As in other reproductive technologies, animal models have provided useful information in transferring methods to treat human infertility. With the sheep ovary providing a reliable tool, Baird and associates [39] pioneered the sheep ovarian transplantation model. Using cryopreserved–thawed ovarian cortical strips, they showed follicular survival and endocrine function, as well as restoration of fertility after transplantation of cryopreserved–thawed ovarian cortical strips.

There are several potential uses of cryopreserved ovarian tissue: transplantation back into the host, in-vitro maturation of primordial follicles and xenografting into a host animal. Each has significant potential problems. If the tissue is transplanted back into the patient, there is a potential for the reintroduction of a cancer nidus. This limits the use of autotransplantation with malignancies that are known to have a predilection for the ovaries, such as leukemia and certain types of breast cancer. Using the present techniques, ovarian tissue is removed from the patient before chemotherapy by laparoscopy. It is frozen in small strips. When the patient is ready for pregnancy they are transplanted back into the patient in a heterotopic or orthotopic site. Ischemic injury to the graft can occur at any of three time intervals in the process: at the time of harvesting the ovarian tissue and preparation before cryopreservation; during the freezing or thawing of the graft; or after transplantation. As this is an avascular graft, ischemic injury to the transplanted tissue results in the loss of virtually the entire growing follicle population and a significant number of primordial follicles [39]. Our studies looked at the effects of pre-freezing ischemia and the cryopreservation process on follicular damage. The studies confirmed that the pre-transplant process did not have a major impact on follicular damage [40]. It is post-transplant ischemia that determines the outcome of the graft.

Oktay and associates [41**] developed three different surgical techniques of ovarian cortical strip transplantation: orthotopic transplant into the pelvis or heterotopically into the arm or abdominal wall. The orthotopic transplant was in a patient with benign disease who required oophorectomy and subsequently underwent transplant of the strips into the pelvis. Ovarian function ceased within the first 9 months [16*]. In two cases of heterotopic transplant to the arm (brachioradialis muscle) with fresh ovarian cortical strips ovarian function ceased after 3 years. In a recent trial by Oktay and colleagues [41**], they transplanted frozen-banked ovarian tissue underneath the lower abdominal skin in a 36-year-old breast cancer survivor. Hormonal functions were restored. Percutaneous oocyte aspiration resulted in the generation of a four-cell embryo that was transferred but no pregnancy occurred [41**]. This one embryo was obtained after multiple cycles of ovarian stimulation.

As discussed, the main limitation of ovarian tissue cryopreservation and transplantation is the loss of a large fraction of follicles during the initial ischemia after transplantation. For this reason, we proposed the transplantation of a whole ovary using a vascular graft [42]. In an animal trial, we obtained whole ovaries that were cryopreserved and transplanted with a vascular anastomosis to the inferior epigastric vessels [43*]. We clearly showed that there was a restoration of reproductive

function in those animals with a successful anastomosis. One potential effect of the autotransplantation of frozen-thawed ovarian tissue is the induction of anti-ovarian antibodies [44*]. This could potentially impact the fertilization potential of the oocytes obtained from the graft.

Other methods of using cryopreserved ovarian tissue

The in-vitro maturation of primordial follicles obtained from frozen-thawed ovarian cortical strips is a possibility that is not available in the near future. Transplantation studies in immunodeficient mice clearly showed follicle maturation and the completion of meiosis I in preparation for ovulation and potential fertilization [45**]. Concerns regarding viral infections as well as general ethical reservations may limit the use of this option in clinical practice.

Conclusion

There is tremendous interest in the preservation of fertility in cancer survivors. A support group website (<http://www.fertilehope.org>) is now available to help patients. A physician dealing with cancer patients needs to be aware of the options that are available to patients. If there is time, ART with conventional IVF and embryo cryopreservation offers the best clinical outcome. Discussion with the oncologist to design an appropriate protocol for ovarian stimulation is necessary. Ovarian transposition is another excellent option if radiotherapy alone is used. Laparoscopy is clearly the technique of choice. Some options, such as treatment with GnRH agonists, are popular but are not supported by randomized clinical trials. Finally, ovarian tissue banking offers hope but is still considered experimental.

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