

# Fertility preservation in cancer patients

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Fertility preservation is an important but often neglected need of cancer patients. There are several options available but many are considered experimental or are unproven. Most require interventions that can postpone initiation of cancer treatment. The options include surgical procedures to move the ovaries out of the path of a radiation field, prophylactic medical therapy with gonadotropin-releasing hormone agonists and storage of gametes, embryos or gonadal tissue. Embryo freezing after conventional *in vitro* fertilization has a high success rate. Experimental fertility preservation procedures such as oocyte freezing and ovarian tissue cryopreservation/transplantation should be offered to cancer patients only under institutional review board oversight. Laparoscopic ovarian transposition is a viable option if radiotherapy is to be used alone. Oncologists, reproductive endocrinologists and other healthcare professionals should be part of a multidisciplinary team that offers cancer patients options to preserve their reproductive potential if they desire to do so.

The number of reproductive-age women with previously treated cancers is increasing in part due to more effective cancer therapy. However these therapies can be associated with damage to the reproductive organs, in particular the ovaries. This is particularly critical in women due to the lack of clearly identifiable germ-line stem cells, although this view was recently challenged [1]. Consequently, preservation of reproductive function and fertility is an important quality of life issue for those at risk of iatrogenic premature ovarian failure (POF). The reproductive impairments secondary to cancer therapy are not limited to fertility issues but also perinatal disorders. These include but are not limited to early pregnancy loss, premature labor and low birthweight [2].

The lack of appropriate clinical trials to support any specific approach has resulted in empirical rather than evidence-based recommendations to cancer patients. Many approaches have been proposed, as shown in Box 1. The most established strategy is embryo cryopreservation. Experimental alternatives to standard *in vitro* fertilization techniques include oocyte and ovarian tissue cryopreservation.

Ovarian transposition involves moving the ovary away from the pelvic radiation field. It is a viable option for those receiving radiotherapy alone to treat a variety of pelvic malignancies. When those patients are to receive additional gonadotoxic chemotherapy, transposition is no longer an option.

The ideal approach is to give a medication before starting chemotherapy that would render the ovary resistant to chemotherapy-induced gonadotoxicity. Although many chemoprotective agents have been tried concomitantly with chemotherapy, only indirect evidence supports their use. The following review will evaluate the currently available evidence on the different fertility preservation options.

## Indications for offering fertility preservation options

Candidates for fertility preservation options include patients undergoing gonadotoxic chemotherapy for the treatment of a variety of malignant and nonmalignant conditions. Some immune disorders such as lupus are also treated with chemotherapeutic agents. Hematopoietic malignancies, particularly lymphomas, are probably one of the most common indications [3]. Treatments of other malignancies such as musculoskeletal, neurological and solid-organ cancers also increase the risk of POF. Moreover, chemotherapy prior to bone marrow transplantation and the recently adopted umbilical-cord stem cell transplantation are increasingly recognized causes of iatrogenic POF. In addition, cervical, anorectal and vaginal cancers are common pelvic malignancies treated with multiagent chemotherapy with possible long-term reproductive consequences. A total of 50% of the 13,000 newly diagnosed cervical cancer cases in the USA were under the age of 35 years [4].

**Keywords:** fertility preservation, oocyte and ovarian tissue cryopreservation, premature ovarian failure

future  
medicine

**Box 1. Fertility preservation options according to the level of the available evidence.****Evidence-based options**

- None

**Most established option**

- Embryo cryopreservation

**Experimental options**

- Oocyte cryopreservation
- Ovarian tissue cryopreservation
- Autotransplantation (orthotopic or heterotopic)
- *In vitro* maturation
- Xenografting

**Options for patients undergoing gonadotoxic radiotherapy**

- Ovarian transposition
- Potential medical options
- Pharmacological protection
- Gonadotrophin-releasing hormone analogs
- Oral contraceptive pills
- Progesterone
- Sphingosine-1-phosphate

Breast cancer is the most common malignancy of reproductive-age women [5]. An estimated 15% of all breast cancer cases occur in women younger than 40 years of age [6]. It is particularly challenging due to the estrogen sensitivity of the tumor, which makes the use of infertility medication that raises estrogen into the supraphysiological range worrisome.

**Determinants of iatrogenic premature ovarian failure**

The extent of fertility failure after chemotherapy is very difficult to predict. Recently, case reports of pregnancies after chemotherapy thought to induce permanent sterility have been published [7]. This makes the evaluation of experimental protective approaches difficult, especially when reporting single cases [8]. Furthermore, specific predictions for individual patients are difficult and, therefore, recommendations of therapy should take into consideration these inaccuracies. However, there are some general principles that have been observed in women undergoing chemotherapy and/or radiotherapy.

**Chemotherapy-induced reproductive impairment**

The exact cellular targets of the gonadotoxic agents are unknown. This is probably due to the close structural and functional relationship between granulosa cells and the oocyte. However, functional or structural impairment of

either cell type leads to impairment of the other. In addition, chemotherapeutic agents may impair follicle development and/or deplete primordial follicles.

Generally, destruction of the growing follicles will lead to temporary amenorrhea, while permanent amenorrhea will result if all of the primordial follicles are destroyed. Furthermore, even if some primordial cells survive destruction these may not be as fertile. In other words, the menstrual cycle may return but fertility is permanently damaged [7]. The incidence of chemotherapy-induced POF is variable since ovarian failure is dependant on many factors. The most important determinants of POF are the age of the patient, drug class, cell-cycle specificity and cumulative dose of the drug.

**Age of the patient**

The risk of POF increases with the increasing age of the woman. This is probably due to the relative decrease in the absolute number of primordial follicles left in the ovary as the patient gets older. Consequently, a 70% destruction of follicles in a younger patient has a different impact compared with an older patient. Thawed ovarian tissue that was frozen prior to chemotherapy has a higher concentration of primordial follicles in prepubertal patients compared with postpubertal women. In a study on human tissue cryopreservation, Poirrot and colleagues reported that before the age of 10 years there were 350–400 follicles/mm<sup>3</sup>, but between the ages of 15 and 34 years, there were only 30–35 follicles/mm<sup>3</sup> [3]. This dramatic decrease in primordial follicles explains the extreme sensitivity of older reproductive-age women to gonadotoxic insults.

An amenorrhea rate of 89% was reported in patients older than 25 years compared with 25% in those younger than 25 years at the time of treatment for breast cancer that included cyclophosphamide. Similarly, the median age of lymphoma patients who became amenorrheic after a multiagent protocol composed of chlorethamine, vincristine, procarbazine, prednisone, doxorubicin, bleomycin, vinblastine (MOPP/ABV) therapy was higher than that of patients who maintained normal menses (26 vs 20 years) [9].

**Type, cell-cycle specificity & cumulative dose of the gonadotoxic agents**

Chemotherapeutic agents are not equally gonadotoxic. Cell-cycle nonspecific chemotherapeutic agents are considered to be more gonadotoxic than cell-cycle specific ones (Box 2). For instance,

**Box 2. Level of toxicity to the ovary with different chemotherapeutic agents.****High risk**

- Cyclophosphamide
- Chlorambucil
- Melphalan
- Nitrogen mustard
- Procarbazine

**Intermediate risk**

- Cisplatin
- Carboplatin
- Doxorubicin

**Low risk**

- Methotrexate
- 5-fluorouracil
- Vincristine
- Vinblastine
- Bleomycin
- Actinomycin D

*Adapted from [40].*

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protocols that include the cell-cycle nonspecific alkylating agents such as cyclophosphamide are considered to be the most gonadotoxic. Older patients who received high-dose alkylating agent therapy or pelvic or total-body irradiation are at particularly high risk for POF after treatment for cancer. Most young patients with Hodgkin's disease treated with multiagent chemotherapy and radiation to a field that does not include the ovaries will be fertile, albeit with a shorter fertility window compared with age-matched controls [10]. However, spontaneous resumption of ovarian function and even spontaneous conception could happen after multiple courses of chemotherapy combined with pelvic irradiation [7].

#### **Radiotherapy-induced reproductive impairment**

A wide variety of reproductive-age malignancies are primarily treated by radiotherapy. For instance cervical, vaginal and anorectal cancers, some germ-cell tumors, Hodgkin's disease and CNS tumors are primarily treated by radiotherapy. The extent of the pelvic radiotherapy-induced damage is not limited to the ovary but it also includes uterine damage.

#### **Radiotherapy-induced ovarian impairment**

Follicles at various stages of development are particularly vulnerable to DNA damage from ionizing radiation. Pelvic irradiation also

results in ovarian atrophy and a marked reduction of the follicular reserve [11]. In addition, oocytes are probably the most sensitive cellular component of the ovary to irradiation. Pyknosis, chromosome condensation, disruption of the nuclear envelope and cytoplasmic vacuolization are the most prominent molecular changes inflicted by radiotherapy that are observed in oocytes.

Similar to chemotherapy, the extent of radiotherapy-induced reproductive impairment is dependant on many confounders. The most important of these are the patient's age, dose of irradiation and possibly the fractionation of the total dose. A single dose is more gonadotoxic compared with fractionated doses [12]. A dose-dependant reduction in the primordial follicle pool was observed upon exposing the ovary to radiotherapy [13]. The irradiation threshold for POF was found to be approximately 300 cGy, as only 13% of studied women experienced POF below 300 cGy compared with 63% above that threshold value [14]. It was also estimated that a dose of less than 2 Gy was enough to destroy 50% of the oocyte population [15]. A single dose of 6.5–8.0 Gy will cause permanent ovarian failure in most postpubertal women. When the ovaries are not directly in the radiation field, it is important to calculate the scatter dose in order to assess how much radiation will be delivered to the ovaries. The extent of ovarian damage is accentuated by the addition of chemotherapy, a trend found with many protocols in the treatment of malignancies [16].

#### **Radiotherapy-induced uterine impairment**

Cancer survivors treated with total-body irradiation are at risk for impaired uterine growth and blood flow, early pregnancy loss and premature labor if pregnancy is achieved. Despite standard estrogen replacement, the uterine volume of these women is reduced to half of its normal adult size. In addition, pelvic irradiation damages the uterine musculature and vasculature. The uterine volume in these women correlates with the age at which radiation was received. It is not known whether standard regimens of estrogen-replacement therapy are sufficient to facilitate uterine growth in adolescent women treated with total-body irradiation in childhood. Exogenous estrogen therapy may restore the uterine volume but will not guarantee a successful pregnancy outcome. Consequently, pregnancies in survivors of childhood cancer should be managed as high risk [17–19].

### Assessment of ovarian reserve

Documentation of POF or decreased fertility potential in patients receiving gonadotoxic chemotherapy or radiotherapy is important not only for diagnostic purposes but for predicting the success rates of specific infertility treatments. Decreased fertility potential due to a potential ovarian problem is referred to as 'decreased ovarian reserve'. There are no ideal markers to predict chemotherapy-induced gonadal damage. Clearly if the serum follicle-stimulating hormone (FSH) measurement is in the menopausal range, then fertility is compromised, although recovery and pregnancies have been reported. If there are regular menstrual cycles then an assessment of ovarian reserve is necessary.

A variety of markers have been tested. Day-3 serum FSH levels were shown to be elevated in cancer survivors with regular menstrual cycles compared with those control patients that did not have chemotherapy. This reflects ovarian damage that is closely linked to the oocyte or the granulosa cells that produce inhibin. Inhibin is a peptide hormone produced by granulosa cells, which has a negative feedback effect on the pituitary that results in decreased FSH levels. Due to the intimate relationship between granulosa cells and the oocyte, it was proposed that decreased inhibin levels and increased serum FSH levels reflect damage to the oocyte-granulosa complex.

Another approach is to assess the response of the ovary to stimulation. Clomiphene citrate, a synthetic anti-estrogen, is administered for 5 days. The estrogen and serum FSH response are measured before and after administration of the drug. The response to this test, known as the clomiphene challenge test, is a reflection of the ovarian reserve.

Gonadotoxic chemotherapy is associated with a transient suppression of inhibin B in prepubertal girls. Inhibin B, together with sensitive measurements of FSH, was proposed as a potential marker of the gonadotoxic effects of cancer chemotherapy in prepubertal girls [20]. Similarly, serum levels of FSH and luteinizing hormone (LH) rise progressively within 4–8 weeks following radiation exposure along with a decline in serum estradiol levels in patients receiving pelvic radiotherapy.

Anti-Müllerian hormone (AMH), a peptide produced by the granulosa cells, is a more sensitive indicator of ovarian damage. Serum levels are low in patients with ovarian damage [21]. The test can be performed any time in the cycle.

Although a sensitive marker of ovarian damage, the test is not generally available and further validation is necessary.

Another determinant of ovarian reserve is the antral follicle count (AFC) [22]. It is defined as the number of follicles smaller than 10 mm in diameter detected by ultrasound in the early follicular phase. There is an inverse relationship between a woman's age and the AFC. The number of antral follicles originating from the cohort of growing follicles also correlates with the number of primordial follicles, or ovarian reserve. It is a promising tool in the estimation of ovarian reserve. In conclusion, although specific data are needed to find out the best method of ovarian-reserve screening in cancer survivors, several markers are available to offer patients some appreciation of their fertility potential after chemotherapy.

### Fertility-preservation options

Many fertility-preservation options have been tried in patients at risk of iatrogenic POF. None of them has been evaluated in a prospective, randomized, controlled trial. Many of the available techniques are promising but are still highly experimental.

#### *In vitro fertilization with embryo cryopreservation*

Embryo cryopreservation is the only option that has long-term data regarding the outcome of children born from these procedures and has an excellent success rate. It is the best option for patients with a partner. However, it might not be acceptable to prepubertal, adolescent girls and women without a partner. The post-thaw survival rate of embryos is 35–90%, implantation rates are 8–30% and cumulative pregnancy rates can be over 60% [23]. A recent Society for Assisted Reproductive Technology (SART) report demonstrated that the delivery rates per frozen embryo transfer are approximately 18.6% [24].

The use of this option as well as oocyte cryopreservation entails controlled ovarian hyperstimulation (COH) to obtain a suitable number of oocytes or embryos for freezing. The extremely high estradiol levels during COH are particularly challenging in estrogen receptor-positive breast cancer patients. This problem could be dealt with using modified ovarian stimulation protocols.

The typical stimulation protocol used in *in vitro* fertilization (IVF) cycles is designed to obtain a large number of oocytes and is called

the ‘long’ or downregulation protocol. In this protocol, the pituitary gonadotrophs are down regulated with a gonadotropin-releasing hormone (GnRH) agonist and then gonadotropins are started to stimulate the ovary. This typically takes at least 3 weeks. Most oncologists will not allow this period of time before initiating chemotherapy. In breast cancer patients there is an interval of 6 weeks between the surgery and initiation of chemotherapy. Therefore, rather than using the typical protocol where the cycle is downregulated with a GnRH agonist and then stimulation started, a short-flare protocol is used.

In this protocol, the pituitary gonadotrophs are not downregulated. The gonadotropins that stimulate the ovary are started soon after the start of a period. A GnRH agonist or antagonist is then used for a brief period of time to prevent premature ovulation. This protocol usually requires less time to achieve follicle recruitment [25]. This protocol typically requires approximately 10 days.

The avoidance of high levels of estrogens in breast cancer patients has resulted in implementing alternative protocols that will not increase estrogen levels excessively. Some centers offer natural-cycle (unstimulated) IVF. A single oocyte is usually aspirated. However, the high cancellation rates and the very low pregnancy rates make this approach impractical and less appealing [26,27].

Tamoxifen, a nonsteroidal anti-estrogen, has also been used for ovarian stimulation and IVF in breast cancer survivors. Using tamoxifen as an ovulation-induction agent, a significantly higher numbers of mature oocytes, peak estradiol and embryos were observed compared with natural-cycle IVF [27]. However, pregnancy rates were still low.

There is a growing body of evidence regarding the safety and success of stimulating the ovary with the aromatase inhibitors, such as letrozole, with or without gonadotrophins in breast cancer patients undergoing IVF. The use of this agent allows estrogen levels to be lower but simultaneously obtains more oocytes and consequently embryos to cryopreserve [28,29].

#### **Oocyte cryopreservation**

Mature or immature oocyte cryopreservation may be the only option in women without a partner. As the oocyte subcellular organelles are more complex and perhaps more sensitive to thermal injury than in pre-implantation embryos, using this approach is more technically

challenging [30]. Recent studies on the outcome of frozen–thawed oocytes suggest increased success rates [31–33].

In addition, the duration of oocyte storage does not seem to interfere with oocyte survival as pregnancies occurred even after several years of gamete cryopreservation in liquid nitrogen in cancer patients [34]. Moreover, with the newly adopted vitrification protocols, the results of oocyte freezing programs are expected to be even better [33].

The practice committee of the American Society for Reproductive Medicine (ASRM) recently concluded that despite the limited number of established pregnancies and deliveries resulting from cryopreserved oocytes, no increase in chromosomal abnormalities, birth defects or developmental deficits have been noted in children born from cryopreserved oocytes to date. The option of oocyte cryopreservation should presently be considered an experimental technique only to be performed under investigational protocol under the auspices of an institutional review board (IRB) [35].

#### **In vitro maturation**

The most important limitation of all the discussed techniques is time, and that may not be available. The reason that it takes time is that the best results are achieved with a mature oocyte. However, the maturation of oocytes that are relatively immature in the laboratory may eliminate this limitation. Typically, a metaphase II oocyte is obtained from IVF for fertilization. However, antral follicles that are observed on ultrasound may be obtained with minimal delay and matured *in vitro*. *In vitro* maturation (IVM) is currently successful in patients with polycystic ovarian syndrome (PCOS). Its use as a fertility-preservation option is dependent on finding the suitable IVM environment. So far, success with IVM other than in PCOS patients is very limited [39]. However the potential is enormous since patients could be seen one day and oocytes could potentially be aspirated the next day.

#### **Ovarian tissue cryopreservation**

After the initial successful animal experiments [36,37], ovarian tissue cryopreservation and transplantation is becoming an increasingly recognized experimental option to preserve fertility in women with threatened reproductive potential [38]. The cryopreserved–thawed ovarian tissue could be subsequently used for transplantation back into the host, IVM of primordial follicles or xenografting into a host animal [39].

The only type of follicle to survive the freeze–thaw process is a primordial follicle. Therefore, IVM in this case is of primordial follicles not antral follicles. The difference in scale to mature these oocytes, days for an antral follicle and months for a primordial follicle, makes the use of IVM for cryopreserved tissue impossible in the immediate future.

The main limitation for autotransplantation is the potential for reintroduction of cancer cells in malignancies that are known to have a predilection for the ovaries such as leukemias and, potentially, breast cancer. The cryopreserved–thawed ovarian tissue could be transplanted orthotopically into the pelvis or heterotopically into the arm or abdominal wall.

Orthotopic transplant has been tried in a patient with benign disease who required oophorectomy and subsequently underwent transplantation of the strips into the pelvis. Ovarian function ceased within the first 9 months [40]. In two other cases of heterotopic transplantation to the arm (brachioradialis muscle) with fresh ovarian cortical strips, ovarian function ceased after 3 years of follow-up. In addition, Oktay and colleagues transplanted cryopreserved–thawed ovarian tissue underneath 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.

Spontaneous pregnancy and delivery was reported 11 months after orthotopic autotransplantation of cryopreserved–thawed ovarian tissue in a Hodgkin's lymphoma patient from whom tissue was collected and cryopreserved before chemotherapy [42]. Another pregnancy was reported after a modified natural IVF cycle following orthotopic autotransplantation of cryopreserved–thawed ovarian cortical strips in a woman with non-Hodgkin's lymphoma [43]. Given that the tissue was transplanted into the native ovary in both cases, the possibility of resumption of native ovarian function could not be excluded. However, the report of a pregnancy after transplantation of fresh ovarian tissue from a fertile woman to her identical twin who had documented ovarian failure clearly points to the feasibility of the technique [44].

With the increasing reports of documented spontaneous pregnancies in women with POF after prolonged courses of gonadotoxic

chemotherapy and/or radiotherapy [7], one should be cautious regarding the exact site of origin of the oocytes that led to the pregnancies in these reports, as natural ovulation could be due to the pre-existing ovarian tissue and not the graft.

Another potential limitation of ovarian tissue cryopreservation and transplantation is the loss of a large fraction of follicles during the initial ischemia after transplantation [37,45]. Research should focus on refinement of the cryopreservation protocols, better cryoprotectants and transplantation techniques that decrease ischemia, particularly the use of vascularized grafts [46–48]. As with oocyte cryopreservation, the ASRM practice committee recommended that ovarian tissue cryopreservation or transplantation procedures should be performed only as experimental procedures under IRB guidelines [35].

Other methods of using cryopreserved ovarian tissue include xenotransplantation in severe combined immunodeficient (SCID) mice [49]. Xenotransplantation of cryopreserved–thawed human ovarian tissue into a suitable animal host is limited by concerns regarding possible viral transmission as well as the ethical constraints surrounding the issue.

#### Ovarian transposition

Ovarian transposition requires moving the ovaries out of the pelvic irradiation field to maintain ovarian function in patients exposed to gonadotoxic radiotherapy. This leads to reduction of the ovarian irradiation dose to approximately 5–10% of that of the *in situ* ovaries [50]. Lateral transposition appears to be more effective than moving the ovary behind the uterus (medial transposition) [51,52]. Lateral ovarian transposition entails dividing the utero-ovarian ligament and mobilizing the ovary with its vascular pedicle to the paracolic gutters. Keeping the vascular pedicles retroperitoneally is essential to avoid tension, torsion or trauma and bowel herniation while the ovaries remain intraperitoneal to reduce cyst formation [53]. Taking a contraceptive agent will reduce the risk of cyst formation further.

Ovarian function is generally preserved if the ovaries are transposed at least 3 cm from the upper border of the field [54]. However, 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. Other technical problems include injury to the ovarian vessels from surgical technique or

radiation [55]. These limitations are often issues of surgical technique, which can be avoided by following some general surgical principles.

Ovarian transposition should be performed laparoscopically prior to the initiation of radiotherapy. An important advantage of laparoscopic ovarian transposition is that radiotherapy can be initiated immediately postoperatively, preventing failure due to the ovaries migrating back to the radiation field [56]. The outpatient nature of the procedure, with more rapid recovery, less discomfort, better cosmetic result and lower cost, are additional advantages to the laparoscopic approach. There are few reports on pregnancy outcomes after pelvic irradiation. However there does not appear to be any increase in stillbirths, low birthweight infants, congenital malformations, abnormal karyotypes or cancer in the offspring of women treated for Hodgkin's disease [57].

### Medical options

Using oral-steroid or GnRH-agonist cotreatment at the time of gonadotoxic therapy was thought to be of value in preventing or ameliorating the extent of chemotherapy and/or radiotherapy-induced gonadotoxicity. Suppressive therapy with a variety of oral steroids such as oral contraceptives [58] or progestins [59] have not been shown to be effective in preventing damage from chemotherapy or radiotherapy.

In the same vein, GnRH-agonist cotreatment was tested. Presumably by suppressing gonadotropin elevation, GnRH agonists inhibit the normal physiological loss of primordial follicles by recruitment and subsequent atresia. In a study of 60 patients with lymphoma, a GnRH agonist was added 7–10 days prior to the initiation of the chemotherapy regimen. The control group consisted of 100 historical patients of similar age range treated with chemotherapy but not a GnRH agonist. The rate of POF was 55% in the chemotherapy-only group versus 5% in the GnRH agonist/chemotherapy group [60].

There are many limitations to this study. First, the retrospective nature of the control group makes the study less convincing. In addition, it cannot be determined whether the lower incidence of ovarian failure is due to GnRH-agonist treatment or to the shorter follow-up. Moreover, primordial follicles initiate follicle growth through an unknown mechanism, which is gonadotropin independent [61]. Finally, there is some controversy regarding the existence of

GnRH-agonist receptors on human ovaries, while GnRH-agonist receptors have clearly been detected in the rat ovary.

GnRH receptors have been identified in malignant tumors of the breast, ovary and endometrium. GnRH agonists and antagonists have been shown to have a direct antiproliferative effect on these cells. This drug is often used by oncologists as it can be initiated immediately and will not delay starting chemotherapy. However, patients should be told of the limited data to support its use.

However, systemic administration of GnRH antagonists caused a significant destruction of primordial follicles compared with controls in a murine animal model. Similar results were obtained whether the antagonists were administered systemically or directly to the ovary. In addition, a GnRH agonist had no effect on primordial follicle numbers by itself but reduced the follicular depletion caused by cyclophosphamide [62]. The value of GnRH agonists needs to be substantiated in a prospective, randomized study with sufficient power.

### Conclusion

So far, embryo cryopreservation is the most successful fertility preservation modality. However, it is not generally acceptable to women without a partner unless they wish to use a sperm donor. Oocyte cryopreservation is gaining popularity as a treatment option. The recent improvements in the post-thaw survival, fertilization, and implantation and pregnancy rates adds to the practical potential of this option. Despite the recent reports of embryo development, pregnancy and delivery after transplantation of cryopreserved–thawed ovarian tissue, the technique remains experimental. Both oocyte and ovarian tissue cryopreservation should be offered after IRB review. Consent needs to be obtained within a research context rather than for therapy or preservation of fertility *per se*. GnRH analogs as a medical protection are simple to use and are commonly offered, although the data are limited. Laparoscopic ovarian transposition is a viable option if radiotherapy is to be used.

Since not enough clinical evidence is available today to recommend most options as 'standard of care', counseling becomes a very important part of the process. Oncologists, gynecologists and reproductive endocrinologists should be aware of the available fertility-preservation strategies. A multidisciplinary

## Executive summary.

### **Indications for offering fertility preservation options**

- Fertility preservation should be offered to all patients of reproductive age that will undergo potentially sterilizing medical or radiation therapy.
- This could include patients with malignant or nonmalignant conditions.

### **Chemotherapy-induced reproductive impairment**

- Chemotherapeutics affect both granulosa cells and the oocyte and impair folliculogenesis and/or deplete primordial follicles.
- The main determinants of chemotherapy-induced premature ovarian failure (POF) are the age of the patient, drug class, cell-cycle specificity and cumulative dose of the drug.
- Chemotherapeutic agents are not equally gonadotoxic.
- Cell-cycle nonspecific agents are considered to be the most gonadotoxic category.

### **Radiotherapy-induced reproductive impairment**

- Follicles at various stages of development are particularly vulnerable to DNA damage from ionizing radiation.
- The most important determinants of POF are patient age, dose of irradiation and fractionation of the total dose.
- It has been estimated that a dose of approximately 2 Gy was enough to destroy 50% of the oocyte population.

### **Radiotherapy-induced uterine impairment**

- Cancer survivors treated with total-body irradiation are at risk for impaired uterine growth and blood flow, early pregnancy loss and premature labor if pregnancy is achieved.

### **Investigations & ovarian reserve screening to predict iatrogenic premature ovarian failure**

- There are no ideal markers to predict chemotherapy-induced gonadal damage.
- Day-3 serum follicle-stimulating hormone, a clomid challenge test and basal antral follicle count are available in all fertility centers. In future, Anti-Müllerian hormone may be available and is superior to presently available tests as it is not dependent on the menstrual cycle.

### **In vitro fertilization with embryo cryopreservation**

- Embryo cryopreservation is the only established option with the best outcome for patients with a partner. However it is not acceptable to prepubertal, adolescent girls and women without a partner.
- Modified ovarian stimulation protocols are frequently needed to avoid a supraphysiological rise of serum estradiol levels, which could be detrimental to the overall prognosis of the patient.

### **Oocyte cryopreservation**

- Mature or immature oocyte cryopreservation may be the only option in women without a partner.
- The option of oocyte cryopreservation presently should be considered an experimental technique performed after institutional review board (IRB) review.

### **Ovarian tissue cryopreservation**

- Spontaneous pregnancy and delivery were reported in Hodgkin's and non-Hodgkin's lymphoma patients from whom tissue was collected and cryopreserved before chemotherapy.
- Ovarian tissue cryopreservation or transplantation procedures should be performed only as experimental procedures after IRB review.
- Other methods of using cryopreserved ovarian tissue include *in vitro* maturation of primordial follicles obtained from the cryopreserved-thawed ovarian cortical tissue or xenotransplantation in suitable animal host.

### **Ovarian transposition**

- Ovarian transposition requires moving the ovaries out of the pelvic irradiation field to maintain ovarian function in patients exposed to gonadotoxic radiotherapy.
- Ovarian transposition could be performed laparoscopically prior to the initiation of radiotherapy.

### **Medical options**

- Gonadotrophin-releasing hormone analogs as a means of medical protection for gonadotoxic chemotherapy are commonly used as they have few significant side effects and are easily initiated.
- The data on its efficacy are limited.

approach is ideal for the management of chemotherapy and/or radiotherapy-induced gonadal failure.

### Future perspective

The dogma of ovarian physiology that germline stem cells (GSCs) do not occur in the postnatal ovary has been recently challenged [1]. This study shows the presence of GSCs in the murine adult ovary and therefore the potential for regeneration of new oocytes if depleted by chemotherapy. If the results of this study are validated, there may be cells available to generate new oocytes in other species. Consequently, if the primordial cells are depleted or destroyed through therapy, new oocytes will be generated. This concept may change entirely the way we understand ovarian dynamics. This area of treatment has implications not only for cancer patients but also all women who wish to delay childbearing. Another strategy for preservation of fertility potential would be in research aimed at the GSC. If future research confirms this preliminary work, there may be the ability to isolate these stem cells and repopulate the ovary as needed.

Apoptosis plays an essential role in germ cell dynamics, both prenatally and postnatally [63,64], and could be activated aberrantly via chemotherapeutic drugs [65,66]. Inhibition of apoptosis signaling events could potentially stop the apoptotic

process and protect the patient from POE. Sphingosine-1-phosphate was investigated as an apoptotic inhibitor. Ceramide is a sphingolipid molecule believed to be an early messenger signaling apoptosis in response to stress. It was documented that oocytes of mice that lacked the enzyme to generate ceramide, acid sphingomyelinase and wild-type mice oocytes that had been treated with sphingosine-1-phosphate therapy, resisted apoptosis induced by doxorubicin [67]. With eventual identification of the molecular and genetic framework of chemotherapy-induced germ-cell death, apoptotic inhibitors may some day play a role in preventing oocyte loss.

The most important advancement that will occur in the next 5–10 years will be the ability to mature follicles in the laboratory and the improvement in oocyte preservation. This IVM is possible with antral follicles but not primordial follicles. This will allow a patient diagnosed with a malignancy to be seen and the procedure performed the next day. No medication or other special preparation will be necessary. The recovery will require a day at most and the patient can proceed without delay with her treatment. The oocytes will be cryopreserved unfertilized. This will eliminate stress for a patient with or without a partner. If the patient decides in the future she wishes to have a family, she can choose her partner.

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