

Where are oncofertility and fertility preservation treatments heading in 2016?

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An improvement in the survival rates of cancer patients and recent advancements in assisted reproductive technologies have led to remarkable progress in oncofertility and fertility preservation treatments. Although there are several available or emerging approaches for fertility preservation, the limited evidence for each strategy is the greatest concern. In this review, we discuss the concerns on currently available options, and propose new approaches for fertility preservation that may be available in the future.

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With recent advances in oncology, the survival rates of cancer patients have greatly improved. Consequently, there is a greater emphasis on the preservation of fertility in cancer survivors. The concept of oncofertility, which unites oncology with fertility, was proposed a decade ago [1]. Together with developments in assisted reproductive technologies (ART), there are several fertility preservation treatments available for patients wishing to have offspring [2].

This review focuses on the future directions of fertility preservation treatments. We discuss the concerns on currently available options and then introduce new approaches for fertility preservation.

An oncofertility algorithm with currently available options

• For male patients

For adults and postpubertal boys, sperm cryopreservation is an established method [3]. There is no option for prepubertal boys, although testicular cryopreservation is being offered at several centers as an experimental technique [4].

• For female patients

For adults and postpubertal girls, oocyte or embryo cryopreservation is an established method [3,5]. If their cancer treatment cannot be postponed for 2 weeks, ovarian tissue cryopreservation is being offered as an experimental technique [6,7]. At the same time, administration of gonadotropin-releasing hormone (GnRH) agonists can be offered for ovarian protection [6]. For prepubertal girls, ovarian tissue cryopreservation is the only option [8]. Against radiation injury, ovarian transposition is an established method [3].

KEYWORDS

• drug delivery systems
• fertility preservation
• *in vitro* follicle growth
• *in vitro* sperm development
• oncofertility • oocyte cryopreservation • ovarian protection • ovarian tissue cryopreservation • uterine transplantation

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Concerns on the currently available options for fertility preservation

• Oocyte & embryo cryopreservation

Vitrification techniques to cryopreserve mature oocytes have already been established. According to the guidelines described by the American Society of Reproductive Medicine (ASRM) in 2013, oocyte cryopreservation with appropriate counseling is recommended for patients facing infertility due to chemotherapy or other gonadotoxic treatments, since this technique should no longer be considered experimental [5]. However, ovarian stimulation for oocyte retrieval, which is also required for oocyte retrieval for embryo cryopreservation, remains to be a concern. This will be discussed in the following paragraph.

Protocols for ovarian stimulation

Many oocytes can be harvested following controlled ovarian stimulation (COS) with gonadotropin administration before starting cancer treatment. However, we should be careful to prevent severe complications during COS, keeping in mind that cancer patients are different from infertile patients in that cancer might be affecting the function of multiple organs. For fertility preservation during cancer treatment, the interruption has to be brief, in order to allow timely commencement of anticancer treatment.

For patients with estrogen-sensitive cancers, the fertility preservation network FertiPROTEKT recommends stimulation combined with letrozole 5 mg daily to prevent a rise in estradiol levels [6]. This aromatase inhibitor is well tolerated, and it does not affect the number of oocytes and embryos retrieved compared with standard protocols, without affecting short-term recurrence of cancer [8].

To reduce the risk of ovarian hyperstimulation syndrome (OHSS), the most serious complication of COS, a GnRH-antagonist protocol is recommended [6]. Final triggering with GnRH agonists instead of human chorionic gonadotropin (hCG) should be considered for patients at risk of OHSS, albeit GnRH agonist trigger sometimes fails to achieve trigger [6,8]. The risk of empty follicle syndrome after GnRH α trigger has been reported to be between 1.4 and 3.5% [9].

For a patient who cannot wait for her next menstrual period but still has 2 weeks before her cancer treatment, random-start COS may be recommended. Random-start COS, which commences at a phase other than the early follicular phase [6,8], does not affect the number of oocytes

and embryos retrieved compared with standard protocols in cancer patients [10]. Furthermore, pregnancy and neonatal outcomes in random-start COS cycles are similar to those in standard COS cycles in infertile patients [11].

• Ovarian tissue cryopreservation

Ovarian tissue cryopreservation has proven to be an effective technique and is now getting popular as an option for carefully selected patients [3,7,12]. This approach is useful for patients whose cancer treatment cannot be postponed for 2 weeks [6]. Furthermore, ovarian tissue cryopreservation is the only option for the preservation of fertility in prepubertal girls. However, the ASRM defines ovarian tissue cryopreservation and transplantation as an experimental approach [7], and following issues are still under debate.

Indications

Ovarian tissue cryopreservation is applicable for all patients who can tolerate anesthesia and surgical procedure. Although there is a risk of reintroducing malignant cells during the transplantation of cryopreserved tissue, the relative risk for most types of cancer is still unknown. However, the risk of reintroducing malignant cells is high for leukemia patients. One systematic review of 289 studies, including follow-up after autotransplantation, detection of cancer cells in ovarian tissue from oncological patients and epidemiological data on ovarian metastases, showed that metastases were common in tissues from leukemia patients but not in those with lymphoma or breast cancer patients [13]. Another systematic review of ovarian tissue from 422 patients estimated the risk to be high for leukemia, moderate for gastrointestinal cancers and low for breast cancer, sarcomas of the bone and connective tissue, and Hodgkin's and non-Hodgkin's lymphoma [14].

Protocols for cryopreservation

Cortical ovarian tissue is cryopreserved for ovarian tissue cryopreservation. All reported live births involved autotransplantation of cryopreserved cortical tissue, although the indications for most cases are premature ovarian insufficiency (POI) without malignancy [15].

Slow freezing versus vitrification

Ovarian tissue can be cryopreserved by slow freezing or vitrification. Slow freezing is the method of choice, because only three live births

have been reported after the autotransplantation of vitrified ovarian tissue [15,16]. However, vitrification is gaining popularity in embryo and oocyte cryopreservation because of good outcomes. Vitrification involves a rapid cooling time, which avoids crystal formation and subsequent damage to ovarian tissue. However, vitrification requires the use of a higher concentration of cryoprotectants, which might cause cellular toxicity and osmotic trauma. Studies showed that vitrification is similar to or better than slow freezing in preserving the morphology of the human ovary and the survival of oocytes [17,18]. Although further studies for outcome are needed to establish the efficacy and safety of vitrification for ovarian tissue, it is a promising method.

Optimizing vitrification protocols

The protocol for vitrification remains to be established, and several investigators are conducting studies to minimize its detrimental effects on ovarian tissue from humans and other primates. Factors that can affect the outcome of vitrification include tissue size, type and concentration of cryoprotectants, exposure time to cryoprotectants, temperature and vitrification device [19–21].

Protocols for transplantation

Orthotopic transplantation versus heterotopic transplantation

Orthotopic transplantation involves transplantation of tissue into the peritoneal cavity such as the ovarian medulla, ovarian fossa or broad ligament, while heterotopic transplantation involves transplantation outside of the peritoneal cavity such as the forearm, abdominal wall or chest wall. To date, all but two (one case of twin) reported live births are after orthotopic transplantation, but in some reports it was not clear if pregnancies came out of the graft. [15,16]. For one case of twin, the anterior abdominal wall was used for heterotopic transplantation [22]. Orthotopic transplantation offers more natural environment including vasculature for ovarian tissue than heterotopic one, and natural conception is expected by ovulation from transplanted ovary. By contrast, heterotopic transplantation offers ease of transplantation and monitoring of the transplanted ovary.

• Ovarian protection

Anticancer agents and radiation therapy induce gonadal damage. Although the effects of different anticancer therapies on fertility have not yet

been determined, the risk of treatment-induced POI is highest with alkylating agents such as cyclophosphamide, busulphan and dacarbazine, as well as with pelvic irradiation. Platinum compounds such as cisplatin and carboplatin, and taxanes, harbor an intermediate risk [3]. As for novel targeted and biological therapies, there are limited data on their effects on gonadal function. However, bevacizumab, an agent that targets VEGF, was reported to have an intermediate risk of POI [3].

Chemotoxicity

There is no consensus on the use of GnRH agonists for the protection of ovaries from the toxic effects of chemotherapy. Recently, the results of two randomized breast cancer trials were reported. The POEMS/S0230 and PROMISE-GIM6 trials examined the efficacy of GnRH agonists in patients with hormone receptor-negative and -positive breast cancer, respectively. Both trials reported a lower incidence of chemotherapy-induced POI and a higher rate of pregnancy with GnRH agonist use than with chemotherapy alone [23,24]. In addition, a recent meta-analysis of randomized trials confirmed the beneficial effects of GnRH agonists in breast cancer patients [25]. Thus, the use of GnRH agonists for ovarian protection during chemotherapy should be considered for breast cancer patients [12]. The benefits of GnRH agonists for malignancies other than breast cancer are not known [26,27].

Radiation injury

Ovarian transposition, a well-established procedure that moves ovaries out of the radiation field, is recommended for patients receiving pelvic irradiation due to cervical cancer or Hodgkin's disease [28]. However, it is not effective for patients receiving total body irradiation (TBI) during hematopoietic stem cell transplantation (HSCT), and ovarian shielding is recommended for these patients [29]. Given the low recovery rate of ovarian function after TBI [30], ovarian shielding should be taken into consideration, although careful monitoring of a relapse is necessary.

• General concerns

Necessity for accumulating data to evaluate the safety & efficacy of the current options

Although the options for oncofertility and fertility preservation are rapidly increasing in this era of ART, there is little evidence for the safety and

efficacy of oocyte, embryo or ovarian tissue cryopreservation for cancer patients. Most results are from studies on young infertile patients. A global registration system and database, with long-term follow-up data, is urgently needed. Several questions need to be addressed such as what are the effects of different treatments on a patient's long-term survival and ability to achieve live births, and the newborn's short-term and long-term health.

Necessity for establishing oncofertility networks worldwide

The oncofertility network commenced with the establishment of the Oncofertility Consortium in North America in 2005. Since its establishment, the oncofertility network has expanded worldwide with the goal of introducing new oncofertility treatments [31]. Regional networks also provide information and healthcare services to cancer patients and their families on fertility preservation, as well as counsel to clinicians and healthcare providers. Through these continuous efforts, oncofertility and fertility preservation treatments are available for most cancer patients wishing to preserve their fertility.

Novel techniques for fertility preservation

• *In vitro* development of sperm from cryopreserved testes

Sperm cryopreservation is the only option available for males wishing to preserve their fertility [3]. However, sperm cryopreservation is not an option for prepubertal boys in which spermatogenesis has not yet started. Testicular cryopreservation, on the other hand, is appropriate for prepubertal boys [32]. Presently, there are no reported human live births originating from sperm obtained from cryopreserved testes [33]. Although sperm can be obtained by grafting thawed testes [34] or transplanting spermatogonial stem cells into testes [35,36] in nonhuman primates, there is a risk of reintroducing malignant cells. These procedures are also quite invasive to patients. Sperm can be harvested from cryopreserved mouse testes and cultured *in vitro* to produce healthy offspring [37]. The most important criteria for the successful *in vitro* development of sperm are the culture conditions, including the culture medium used and the incubation temperature [37]. A specialized culture device that utilizes microfluidic techniques to mimic the *in vivo* environment is also required [38]. This approach may benefit prepubertal boys wishing to father offspring in

the future. Presently, this option is being offered at several European and North American centers as an experimental technique [4,39–41].

• *In vitro* growth & maturation of follicles

Ovarian tissue cryopreservation and transplantation are the only options available for prepubertal girls. However, there is a risk of reintroducing malignant cells with these procedures. To overcome the limitations of transplanting cryopreserved tissue, immature follicles can be harvested from ovarian tissue and matured *in vitro* to produce mature oocytes for fertilization. Several techniques achieved the development of antral follicles *in vitro* from secondary follicles harvested from primate and human ovarian tissues [42–49]. Interestingly, recent studies on mouse secondary follicles revealed that an even very simple method using a Matrigel drop and activin A supplementation induced follicle growth, resulting in the production of healthy offspring [50]. However, the performance of *in vitro* follicular culture system largely differs across species. More immature follicles such as primary and primordial follicles, which are the most abundant in the ovarian cortex, require rigid scaffolds to maintain their follicular structure for a considerably longer culture period. Encapsulation into alginate hydrogels helps to maintain their structure and promotes their development into antral follicles for non-human primates [51]. Encapsulation into alginate gels, however, cannot promote the growth of primary and primordial follicles isolated from human ovarian tissue [52]. Instead, growth and differentiation of these follicles within ovarian cortical tissue encapsulated by alginate hydrogels successfully achieved induction of antral follicles *in vitro* [52]. Moreover, a recent study reported the *in vitro* development of human metaphase II (MII) oocytes with this alginate hydrogel-based encapsulation method [53]. One promising way to grow primordial and primary follicles into mature follicles *in vitro* is to culture these follicles within hydrogel-encapsulated ovarian cortical tissue. Another interesting way is to transplant primordial follicles within artificial biomaterial grafts. A recent paper reported the successful production of offspring in mouse infertile model following transplantation of primordial follicles within artificial biomaterial grafts [54]. Several investigators are studying whether deleterious epigenetic events occur in cultured follicles [55,56].

- **Generating germ cells *in vitro* from pluripotent stem cells**

Recent advances in regenerative medicine and reproductive engineering have made it possible to generate germ cells *in vitro* from rodent induced pluripotent stem (iPS) and embryonic stem (ES) cells [57–59]. However, many technical and ethical issues remain to be addressed before this method can be applied to humans.

- **Novel strategies for gonadal protection**

There are several approaches in development to minimize gonadotoxicity due to chemotherapy or radiation therapy. One strategy involves the development of agents to reduce gonadal damage, while another approach involves the development of drug delivery systems (DDS) to reduce the exposure of gonads to anticancer agents.

Potential agents that reduce gonadal damage

Sphingosine-1-phosphate

Irradiation and most anticancer agents induce DNA damage, resulting in the activation of apoptotic pathways. Sphingosine-1-phosphate (S1P) is an inhibitor of the ceramide-promoted apoptotic pathway [60]. Studies performed in mice reported the *in vivo* protective effect of S1P on cyclophosphamide-induced ovarian damage in human ovarian tissue xenografted into mice [61,62] and on dacarbazine-induced ovarian damage in mice [63]. The *in vivo* protective effects of S1P and the S1P mimetic FTY720 on radiation-induced ovarian damage were also reported in rodents [64,65] and primates, as well as human ovarian tissue xenografted into mice [66]. Although S1P is a promising protective agent, especially against radiation-induced ovarian damage, one big drawback is that it requires continuous administration or local injection [60]. In addition, it is not known whether S1P can attenuate the therapeutic effects of irradiation and anticancer agents by blocking apoptotic pathways [67].

Imatinib

Imatinib (Gleevec), a tyrosine kinase inhibitor, is an anticancer agent that inhibits the c-Abl apoptotic pathway [58]. c-Abl activates TAp63, a transcription factor that promotes the apoptosis of primordial follicles induced by DNA damage [68]. Imatinib also exhibits protective effects on cisplatin-induced ovarian damage in mice [68,69]. Additional studies are needed to determine the safety and efficacy of imatinib.

Ammonium trichloro (dioxoethylene-o,o') tellurate (AS101)

The ovotoxic effects induced by anticancer agents are not only due to apoptosis; they are also due to 'burn out' of the ovarian reserve [70]. 'Burn out' involves the activation of dormant follicles by anticancer agents such as cyclophosphamide and cisplatin, resulting in the depletion of the ovarian reserve. The activation of dormant follicles is mediated by the PI3K/PTEN/Akt pathway [71,72]. Ammonium trichloro (dioxoethylene-o,o') tellurate (AS101) is a small, nontoxic tellurium (IV) compound that affects the activation of the PI3K/PTEN/Akt pathway. AS101 is also a potent immunomodulator that mediates anti-inflammation, chemosensitizes cancer cells and prevents chemotherapy-induced toxicity [73]. The protective effects of AS101 on cyclophosphamide-induced ovarian damage were reported in mice [67]. Additional studies are needed to determine the safety and efficacy of AS101.

DDS to reduce chemotherapeutic-induced gonadotoxicity

Recent advances in DDS are rapidly changing the ways in which drugs are delivered to patients; however, it is not clear whether these systems will be useful in the reduction of gonadotoxicity. These advances enhance the site selectivity and treatment efficacy, and reduce the toxicity of nontargeted organs [74]. Liposomes, hydrogels, and nanoparticles have been investigated as drug carriers. Among the various anticancer agents, doxorubicin, one of the anthracycline antibiotics, which exerts cumulative cardiotoxicity, is one of the most extensively investigated drugs in association with DDS [75]. Currently, several liposome anthracyclines are approved for clinical use. Regarding the prevention of gonadotoxicity, investigation on DDS is still in its infancy. Nano-encapsulated arsenic trioxide, which treats hematological malignancies, was less deleterious to ovarian function in a murine lymphoma model, and more efficacious against lymphoma [76].

- **Uterine transplantation**

Uterine transplantation is the last resort for women without functional uterus, wishing to have offspring. There are 11 reported cases of uterine transplantation; seven of these resulted in pregnancies, whereas five of these resulted in live births [77]. The uterus was absent in the majority

of these patients due to the Mayer-Rokitansky-Küster-Hauser syndrome. In one patient, the uterus was lost due to cervical cancer. Although remarkable advancements have been made in uterine transplantation, there are many technical, safety and ethical issues that must be addressed before this method becomes widely available [78]. The greatest disadvantage of uterine transplantation is that long-term use of immunosuppressive agents can induce a relapse of malignancy in cancer patients. In general, the incidence of malignancy is higher in patients receiving organ transplants than in those not receiving transplants. Especially, the incidence of cancers with infectious cause, such as cervical cancer, increased significantly in transplant recipients [79].

Conclusion

New oncofertility and fertility preservation treatments will continue to be introduced. However, additional data are needed on the safety and efficacy of these fertility preserving options in patients with and without malignancies. It is also important to address all ethical issues associating with these approaches [80]. The goal is to make oncofertility and fertility preservation treatments available to all patients requiring them.

Future perspective

Recent advances in ovarian tissue cryopreservation and *in vitro* follicular growth may change the standards of oncofertility treatment in 5–10 years. The protocol for the

EXECUTIVE SUMMARY

Concerns on the currently available options for fertility preservation

- Oocyte and embryo cryopreservation with vitrification is the standard method. Protocols for ovarian stimulation, which would not affect cancer treatment and cancer status, should be applied. A protocol combined with letrozol is useful for estrogen-sensitive cancers and a random-start controlled ovarian stimulation is effective to shorten the time to simulation.
- Ovarian tissue cryopreservation and transplantation are still at an experimental stage. The indication of these techniques especially for leukemia patients is controversial, because of the lack of data on the risk of reintroducing malignant cells. These techniques represent the only options for prepubertal girls. Vitrification is a promising technique for ovarian tissue cryopreservation. Techniques for transplantation are under development.
- To protect the ovary from anticancer agents, gonadotropin-releasing hormone agonists should be considered, especially for breast cancer patients. The benefits of gonadotropin-releasing hormone agonists for other malignancies remains to be determined. For total body irradiation, the ovary should be shielded.
- Additional data are needed on the safety and efficacy of these fertility-preserving options in patients with and without malignancies. Continuous efforts to expand oncofertility networks are also required for fertility preservation to be available to women globally.

Novel techniques for fertility preservation

- Techniques for the *in vitro* development of sperm from cryopreserved tissue are steadily progressing. Healthy mice can be produced using sperm developed *in vitro* from cryopreserved testes. Presently, this is the only fertility-preserving option available for prepubertal boys. It should be considered in anticipation of a human application.
- Techniques for the growth and maturation of *in vitro* follicles are also steadily progressing. Metaphase II mature oocytes were successfully developed from primordial follicles derived from human cryopreserved ovarian tissue. Studies are needed to determine the occurrence of epigenetic events in oocytes *in vitro*.
- Producing germ cells *in vitro* from pluripotent stem cells still harbors many technical and ethical issues in humans, although it has been achieved in rodents.
- Agents that can reduce gonadal damage such as sphingosine-1-phosphate, imatinib, and AS101 are under investigation. It is not completely clear whether the anti-apoptotic agents sphingosine-1-phosphate and imatinib can attenuate the therapeutic effects of anticancer agents. Gonadal damage can also be decreased by using novel drug delivery systems that target a specific organ.
- There are many technical, safety and ethical issues that must be addressed before uterine transplantation is widely available. The greatest risk after uterine transplantation is that of relapse of malignancy due to the long-term use of immunosuppressive agents.

cryopreservation of ovarian tissue will be established, and either slow freezing or vitrification will be the method of choice. We believe that vitrification will be commonly used for cryopreservation of ovarian tissue like for oocyte and embryo cryopreservation. *In vitro* techniques will also be established for the development of human mature oocytes from primordial follicles recovered from cryopreserved ovarian tissue. This technique will exclude the possibility of reintroducing malignant cells during the transplantation of thawed ovarian tissues. We may also witness the development of non-human primate or human sperm *in vitro* and the application of testicular cryopreservation in prepubertal boys. The use of GnRH agonists for malignancies other than breast cancer may also be established. Furthermore, gonadal damage induced by anticancer agents may be eliminated by novel DDS. Technically, generating human

germ cells from pluripotent stem cells might be achieved and uterine transplantation would also be established, although we also anticipate that there will be much discussion on the ethical concerns behind these approaches.

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