

Future Fertility of Women Cancer Survivors: The Progress in Fertility Preservation

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Abstract

Diminished ovarian reserve (DOR) has devastating implications for the affected female in terms of quality of life, fertility, fecundity and overall well-being. DOR can be exacerbated by the use of chemotherapy and gynaecological surgeries. Analysis of the gonadotoxicity and efficacy of chemotherapeutic regimens currently available and the techniques for gynaecological surgeries, allows for the most appropriate regimen or technique to be selected following a risk-benefit ratio analysis. This should be done in conjunction with fertility preservation strategies including cryopreservation, ovarian transposition and suppression, and pharmacological agents. New approaches using fertoprotective agents need to be evaluated in order to keep up with the demand for oncofertility. This ensures the least possible damage to the ovarian environment and the utmost preservation of the follicular pool.

Keywords: *Oncofertility; Fertility Preservation; Women; Chemotherapy; Ovarian Cancer; Follicular Pool*

Introduction

With the increased survival rates of female cancer patients, the quality of life after treatment is improving considerably, with patients who have had adequate treatment and follow-up even after having suffered from hormone-sensitive cancers considering pregnancy [1]. Oncofertility increases the quality of life of patients who survive cancer by aiming to prevent pre-mature ovarian failure or even infertility [2].

Up till recently, the only fertility preservation strategies for pubertal girls and for post-pubertal women were embryo cryopreservation, prior to the initiation of gonadotoxic chemotherapy using sperm donor in the latter or ovarian transposition in pubertal women undergoing pelvic irradiation [3]. Nowadays, new methods, like oocyte harvesting and oocyte cryopreservation, are being carried out [3-5]. In pre-pubertal girls, the only option remains ovarian tissue cryopreservation [6,7] because ovarian stimulation and subsequent oocyte cryopreservation may induce ovarian hyperstimulation syndrome (OHSS) and is thus considered inappropriate [1,8,9].

Aim of the Study

The aim of this review is to discuss the most commonly used stimulation protocols whilst highlighting the new emerging strategies for fertility preservation.

Cryopreservation

Cryopreservation is a procedure that can be performed on ovarian tissue, embryos and also gametes prior to initiating chemotherapy [8]. Cryopreservation can be done via two methods: slow freezing or vitrification. Vitrification involves rapid cooling of tissues to prevent crystal formation [7,10]. Despite the risk of cellular toxicity and osmotic trauma following the use of cryoprotectants in vitrification [4], the latter led to an increase of cryopreservation [11].

Oocyte cryopreservation is usually preceded by two weeks of ovarian stimulation and protocols differ according to the cause of infertility [10]. One option for controlled ovarian stimulation includes using a GnRH antagonist and gonadotropins concomitantly so as to prevent OHSS. In patients with oestrogen/endocrine-sensitive cancers, letrozole or tamoxifen can be used to prevent an increase in oestrogen levels [4,10]. With vitrification, success rates for oocyte cryopreservation have improved [1,12-14] leading to increased clinical pregnancy rates (CPR) and live births achieved when compared to slow-freezing [10,13,15,16] after conception with fresh oocytes [13,17].

Similarly, embryo cryopreservation is also a reliable, safe and effective procedure especially with vitrification [7]. However, it poses ethical issues [8,10] and success is age-dependent since the highest live-birth rates occur in patients less than 35 years old [18].

Ovarian tissue cryopreservation is a standardised, invasive procedure [4] where multiple strips of ovarian tissue are taken from the ovarian cortex from one ovary [2], with the amount of tissue harvested being relative to the estimated risk of ovarian failure [7]. Transplantation of the ovarian tissue post-cancer increases the likelihood for restoration of normal ovarian tissue activity [2,5]. Overall the complication rates are low [6] but, re-implantation of malignant cells may occur [6,8] especially after haematologic malignancies. Transplanting ovarian follicles instead of the entire ovary may reduce the risk of re-introducing cancer cells [9,19]. Transplantation can be done in two ways: orthotopic involving the transplantation of ovarian tissue (medulla, fossa or broad ligament) inside the peritoneal cavity; or heterotopic, involving the transplantation of ovarian tissue outside the peritoneal cavity (abdominal wall, chest wall or forearm) [4,20]. Orthotopic transplantation is usually more successful than heterotopic [8] provided the fallopian tubes are intact [20] as it provides an optimal environment for follicular growth than heterotopic transplantation [7]. Provided that the proper preservation of ovarian tissue is carried out, transplantation will ensure ovarian function for 4 - 7 years [7].

This technique of fertility preservation has however been surrounded by controversies with regards to chromosomal abnormalities as evidence suggests that cryopreservation may lead to alterations in cell membranes, DNA integrity and aneuploidies [21]. Cryopreserved embryos by vitrification showed no increase in prevalence of aneuploidy and chromosome abnormalities [22,23]. This could also be extrapolated for embryos derived from cryopreserved oocytes whereby although there is impaired blastulation, the rate of euploidy is equivalent to that of blastocysts derived from fresh oocytes [24]. In this regard, vitrification has been regarded as the safest technique of cryopreservation [21,22,25]. Cryopreservation regulations vary in different countries [11].

Ovarian transposition

Also known as oophorexy, this procedure is used in patients prior to pelvic radiotherapy, whereby the ovaries are laparoscopically moved out of the radiation field usually by fixing them to the anterolateral abdominal wall [4,7]. Performing oophorexy before radiotherapy leads to ovarian preservation in 85% of patients less than 40 years of age with previous normal ovarian reserve and leads to 5 - 10% decrease ovarian exposure to radiation [18].

Ovarian suppression

Gonadotropin releasing hormone (GnRH) agonists/analogues, the first agents used in ovo-protection for chemotherapy [26], inhibit the hypothalamic-pituitary-ovarian (HPO) axis thus reducing the susceptibility to and protecting the ovary from chemotherapeutic toxicity [27]. The mode of action of GnRH agonists is not biologically plausible for ovarian function protection because it induces a pre-pubertal state and therefore if this ovarian suppression was indeed protective, then children would be immune to the gonadotoxic effects of chemo-

therapy. Also, the ovarian suppression caused by GnRH agonists does not protect DNA double strand breaks caused by certain anti-cancer agents [28].

Therefore, fertility outcomes of these agents are controversial and inconsistent [7,29-31]. Some researchers claim that there is no effect on pregnancy rates [32] and others claim that such agents are beneficial on the rate of pregnancy and ovarian function [33]. Thus, GnRH agonists may provide a modest protection for ovarian function especially in patients less than 40 years of age [31].

Pharmacologic protection

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S-1-P) reduces follicular apoptosis by inhibiting the ceramide-induced apoptotic pathway namely the sphingomyelin pathway. S-1-P increases the vascular supply, density and angiogenesis in human ovarian tissue xenografted in mice and the apoptosis induced by cisplatin and doxorubicin is eliminated or significantly reduced [34]. Thus, it could be extrapolated that its use in ovarian transplantation reduce the delay in the re-oxygenation of the graft and hence prevent follicular loss [7]. Use of S-1-P on sheep ovaries during cryopreservation reveals its capability to increase the densities and improve the quality of primordial follicles as well as tissue survival in a short time [35]. When applied to human granulosa cells which are exposed to cyclophosphamide, S-1-P activates the Akt pathway which is a cytoprotective effect [36].

S-1-P may be injected directly in the ovary, due to its very short half-life, starting 24 hours prior to giving the chemotherapy and continuing for 72 hours post-chemotherapy administration [26,37]. However, there are two major concerns about this agent: firstly, it is unclear whether it interacts with other chemotherapeutic agents and secondly, the apoptotic block by S-1-P may prevent physiologic apoptosis such as in DNA damaged oocytes [34].

Imatinib

Apart from being a tyrosine-kinase inhibitor used in the cancer treatment, imatinib is also a c-Abl kinase inhibitor which inhibits the activation of apoptotic pathways through TAp63 when used concomitantly with cisplatin [26] but not with other chemotherapeutic agents like doxorubicin [38]. Conflicting evidence suggests that imatinib does not offer protection against cisplatin-induced primordial follicle apoptosis in mice [39] since it may induce follicular apoptosis itself. However, its gonadotoxicity is still unclear and requires further investigation [40].

Anti-mullerian hormone

The administration of supraphysiological doses of anti-Mullerian hormone (AMH) prevents primary ovarian insufficiency by blocking the activation of primordial follicles caused by chemotherapy. In mice, concomitant administration of chemotherapy and AMH provides contraception without modulation of the HPO axis and without disrupting the ovarian reserve [41]. The use of AMH is advantageous because, being an endogenous hormone, the risks for side-effects are less [26].

AS101

AS101 is an immunomodulator, tellurium-based compound [26] and an anti-oxidant [42] that is administered both orally and intravenously [34]. AS101 preserves fertility and ovarian reserve in cyclophosphamide exposed ovaries by different methods. It enhances DNA repair processes in oocytes thus preventing the triggering of follicular activation [7], directly inhibits the PI3K/PTEN/Akt signalling pathway, preventing death of large follicles and maintains the negative feedback inhibition on follicular activation through the release of AMH [43,44]. Inhibition of integrins may also be the way AS101 leads to the downregulation of the PI3K/PTEN/Akt pathway [34]. AS101 maintains the genomic integrity of the oocytes and does not interact with anticancer agents [26].

Granulocyte colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) reduces the destruction of primordial follicles through protection of the vasculature [45] mainly by increasing the microvessel density [46] thus extending the time to ovarian insufficiency after gonadotoxic chemotherapy in mice. The ischaemia and ischaemia/reperfusion injury is prevented with the administration of G-CSF [47] leading to increased AMH levels as well as increased counts of all follicle types [48]. However, such results still need to be produced clinically in humans.

Melatonin

Melatonin is thought to modulate the ovarian damage induced by chemotherapy when administered concomitantly leading to preservation of follicles. In mice, melatonin inhibits the cisplatin-induced phosphorylation of elements within the PTEN/AKT/FOXO3a pathway thus maintaining follicular dormancy and preventing follicle loss [42]. The anti-oxidant effects of melatonin can also be used to protect against radiation-induced damage to the ovary because it inhibits the formation of hydroxyl radicals that cause mitochondrial damage [49]. Although endogenous ovarian melatonin is present it is still not fertoprotective [50]. Therefore, the mechanism by which melatonin protects the ovary from gonadotoxic damage still needs further investigation.

Novel strategies

In-vitro growth and maturation of follicles

With ovarian tissue transplantation, there is a well-known risk that malignant cells may be re-introduced in the patient. This is overcome by a novel strategy where immature follicles are harvested and grown *in-vitro* until maturity within alginate hydrogels [4].

This procedure is challenging because the development of follicles is a multistage process. In humans, only non-growing ovarian follicles have been achieved. If successful in humans, such a procedure may be carried out in girls at all ages [51] but further studies are required.

Germ cells from pluripotent stem cells

The *in-vitro* generation of germ cells is still being investigated in rodents and has not been applied to humans due to many technical and ethical issues [4] namely the xenotransplantation of human stem cells [7]. Germ cells are derived from stem cells or induced pluripotent stem cells (iPSC) and embryonic stem cells (ESC). While in humans it is still not possible to differentiate human embryonic stem cells into oocytes, the attainment of autologous oocytes from human induced pluripotent stem cells is already being studied [52].

In mice and humans, the presence of oocyte stem cells (OSC) leads to the formation of oocytes and early follicle structures, respectively [51]. However, OSCs in humans are scarce [7,51].

Uterine transplantation

Uterine transplantation is carried out in women who have lost their uterine function [4,51]. This procedure primarily helps women born with congenital absence of the uterus but similarly could be extrapolated to cancer patients [51]. Donors of the uterus can either be live or deceased as long as the donor organs have developed to their maximum potential [53]. Subsequently, the uterus is transplanted to the recipient who is given immunosuppressive drugs such as tacrolimus, azathioprine, and corticosteroids [54] so as to minimize or reverse any organ rejection [53]. In 2014, the first live birth was reported in Sweden from a 35 year old woman who had undergone IVF following uterine transplantation [54] and so far, 30 MAKE SURE THIS IS THE MOST UP TO DATE reports of human uterine transplantations have been reported in scientific literature [55]. Being a novel technique more in-depth analysis is required about technical and ethical issues involved [4].

Artificial ovary

The artificial ovary is made up of a scaffold usually a 3D biodegradable material [7], such as alginate, collagen, fibrin, plasma clot or decellularised ovarian extracellular matrix or synthetic polymer like polyethylene glycol, that encapsulates pre-antral follicles [51,52].

Primordial pre-antral follicles are isolated from the patient and transferred onto the scaffold after which they are cryopreserved. This eliminates the risk of transmission of malignant cells [7]. Such a technique provides a low recovery of follicles but of which almost all are viable [56].

Conclusion

Cryopreservation still remains the most commonly used fertility preservation strategy with vitrification being the preferred method to carry out such procedure due to it being relatively safe and efficacious. Other fertility preservation strategies along with fertoprotective agents present more challenges, are controversial or are delimited by inconsistencies in literature thus requiring further in-depth analysis to corroborate existing data.

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