

Fertility-sparing in cancer patients

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Objective: This review aimed to investigate and summarize the current evidence on fertility-sparing options in cancer patients. **Mechanism:** Fertility preservation methods are becoming popular through the improved prognosis of cancer patients at a younger age and early diagnostic tools. However, currently, more and more women are suffering from iatrogenic ovarian failure and fertility loss because of cancer treatment. Most treatments have been used for hematological malignancies, but different gynecological cancers can be eligible. **Findings in brief:** Fertility preserving strategies such as oocyte and embryo cryopreservation, ovarian tissue preservation, ovarian transposition, and aspiration of ovarian follicles are the methods that could be suggested to the patients. The current knowledge supports oocyte and embryo cryopreservation as feasible, safe, and effective treatment approaches for cancer patients seeking fertility preservation. **Conclusions:** Robust evidence is still needed to prove the effectiveness of cryopreservation of the ovarian tissue and ovarian follicle aspiration approaches since these techniques are still in early their steps.

Keywords

Cryopreservation; Infertility; Organ transplants; Ovarian neoplasms

1. Introduction

Patients with malignancy have an increased life span due to success rates in early diagnosis and satisfactory standards of treatments [1]. It is suggested that 6% of reproductive-age women continue to live with different types of malignancies [2]. Moreover, in some cases, cancers are diagnosed in pregnancy [3–5]. However, the effects of cytotoxic chemotherapy and radiotherapy on fertility have raised significant concerns, such as the burden of ovarian follicles, the fibrosis of the ovarian cortex, the deterioration of ovarian vascularization, and the induction of apoptotic processes of oocytes and granulosa cells [6–8].

In patients who require chemotherapy, the extent of ovarian damage depends on the type, length, and dosing schedule of the anticancer treatment combined with patients' age [9, 10]. Instead, the iatrogenic effects of radiation depend upon the closeness of ovaries to the radiation field [9, 10]. Regarding the radiotherapy effect, 10% of patients who underwent vaginal brachytherapy and 40% of the patients who required external radiation therapy with or without vaginal brachytherapy lost their ovarian function [11–13]. The risk of damage determined by radiation increases with the total dose. A dose lower than 2 Gy is expected to decrease approximately 50% of primordial follicles; a quantity of 5–10 Gy is nearly toxic for all follicles [14]. Anticancer therapies may also affect genetic material and embryonic development [15]. An experimental study was conducted to investigate the cytotoxic effect of cyclophosphamide in an *in vitro* fertilization model. The study reported that the fertilization rate and the embryonic development were significantly reduced, and the percentage of aneuploid embryos was considerably increased compared to controls [15].

Considering the detrimental effects of anticancer therapies on fertility, fertility-preserving strategies have gained importance in cancer patients. Different methods ranging from pharmacological protection to surgical interventions, such as ovarian transposition, oocyte cryopreservation, and embryo and ovarian tissue freezing, have been implemented, combining assisted reproductive techniques and surgical methods [12, 16, 17].

Fertility-sparing strategies have to be personalized to the patient and cancer type [12, 17]. Most treatments have been used for hematological malignancies [11, 18], but different

cancers can be eligible. In this regard, considering the peculiar characteristics of gynecologic pathologies, which even in the case of the benign disease require fertility-sparing approaches [19, 20], fertility-sparing methods are used with specific consideration for the patients with gynecological malignancies, such as cervical, vaginal, ovarian, and uterine cancers [21, 22]. To emphasize the importance of fertility-preserving strategies, an up-to-date guideline published by the American Society of Clinical Oncology suggested that in all women who need gonadotoxic treatments, fertility-preserving options should be considered regardless of age, parity, or prognosis [23]. This review aimed to investigate and summarize the current evidence on fertility-sparing options in cancer patients.

2. Pharmacological fertility-sparing strategies

Chemotherapy has detrimental effects on both primordial follicles and growing follicles, resulting in infertility and premature ovarian failure [6]. The “ovarian protection” therapies preserve ovarian reserve, especially in prepubertal females, compared to other surgical alternatives [23]. Medical therapies have been investigated employing GnRH agonists, Sphingosine-1-phosphate, imatinib, thalidomide, tamoxifen, G-CSF, and AS101 to overcome this unintended burden of anticancer treatments. Nevertheless, these drugs were studied only in rodent models [24]. Only GnRH agonists and Sphingosine-1-phosphate have been used in humans.

2.1 GnRH agonists

GnRH agonists prompt the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland, resulting in the long-term downregulation of gonadotrophin secretion [25, 26]. Different studies assessed the follicle preserving effect of GnRH agonists, particularly in lymphoma and estrogen-receptor-positive breast cancer patients [27]. Various mechanisms have been proposed to explain the follicle preserving effect, such as reducing primordial follicles entering the differentiation stage, the lower recruitment of primordial follicles, and the decrease of apoptosis [25]. Besides, GnRH agonists may lower the vascularity of gonads and cause reduced levels of gonadotoxic agents in the targeted organs. Nevertheless, data on the protective effects of this treatment are questioned due to the heterogeneity of study populations and procedures and the lack of a proven mechanism of action for ovarian protection with GnRH agonist [26–28].

In a study with 257 premenopausal women with operable hormone-receptor-negative breast cancer, patients received standard chemotherapy with the GnRH agonist goserelin [29]. The study reported an ovarian failure rate of 8% in the goserelin plus chemotherapy group and 22% in the chemotherapy-alone group after a 2-year follow-up. The pregnancy rates were also higher in women in the goserelin plus chemotherapy group than in the chemotherapy alone group (21% vs. 11%) [29]. Conversely, a two-center, four-

arm, open-label randomized controlled trial (RCT) of GnRH analog co-treatment (GnRH antagonist and agonist combination) in breast cancer patients undergoing cyclophosphamide chemotherapy alone reported no significant protective effect on ovarian function [30]. In another RCT, no protective effect of GnRH agonists was observed in preserving ovarian reserves after cyclophosphamide, cisplatin, or paclitaxel in co-treatment [24].

2.2 Sphingosine-1-phosphate

The other ovarian-preserving agent, studied in both human and experimental investigations, is Sphingosine-1-phosphate, which inhibits apoptosis via the sphingomyelin pathway. Sphingosine-1-phosphate pretreatment was shown to reduce the radiation-related burden of primordial follicles in rats, primates, and xenografted human ovarian tissue [31]. A translational research study evaluating the protective effect of Sphingosine-1-phosphate on human ovarian tissue concluded that Sphingosine-1-phosphate might promote follicle survival in human ovarian cortical samples in *in vitro* environment. However, there are still concerns regarding the administration route of Sphingosine-1-phosphate, and further human studies are needed [32].

3. Surgical fertility-sparing strategies

Ovarian transposition can be considered for young patients who require pelvic irradiation [27, 33]. The procedure consists of mobilizing ovaries out of the pelvis, keeping gonads out of the radiation field. The adnexal pedicle should be fixed to the peritoneal surface of the lateral abdominal wall, 3–4 cm above the umbilical line [34]. The operation’s essential purpose is to ensure adequate ovarian blood, and metallic clips can be utilized to identify ovaries during radiotherapy.

Apart from surgery-related complications, another primary concern with ovarian transposition is ovarian metastases. Moreover, complicate future oocyte retrieval due to moving ovaries mostly out of the pelvic cavity is an additional concern, although a few studies reported spontaneous conception. Finally, adjuvant chemotherapy and the scattering effect of radiation may cause a decrease in ovarian blood supply, which can be particularly detrimental for patients with advanced age [33].

4. Assisted reproductive techniques as fertility-sparing strategies

4.1 Mature oocyte cryopreservation

Mature oocyte cryopreservation is regularly involved in assisted reproduction. However, in cancer patients, the method is not practicable for prepubertal girls [35]. Moreover, the required fourteen days of ovarian stimulation are the major limitation in reproductive age women, considering that anticancer treatments may require immediate action [36]. For this reason, alternative protocols have been proposed for an “emergent ovarian stimulation”, such as the random [37] and the dual stimulation protocols [35]. The dual

stimulation protocol is a protocol in which ovaries are stimulated in both the follicular and luteal phases of the same cycle, yielding a higher oocyte number [35]. These strategies are practical in young patients who do not require an immediate life-saving chemo or radiation therapy, allowing patients at higher risk of iatrogenic premature ovarian insufficiency (POI) to have a possibility of pregnancy.

Even in cancer patients, age is the primary criterion predicting pregnancy rate and success of this treatment. Cobo *et al.* [38] reported retrospective data on the expected live birth rate per oocyte in women aged ≤ 35 years and ≥ 36 years. There was a 60.5% probability of live birth in patients ≤ 35 years old compared to a rate of 29.7% in patients ≥ 36 years old [38]. Goldman *et al.* [39] evaluated live birth rates of thawed oocytes and found that physicians should educate their patients about the real and probable pregnancy rates considering their age and ovarian reserves. Also, in some studies, the live birth rate per vitrified oocyte was reported around 5.7%, with approximately the need of 10 oocytes for a patient to have a fair chance of pregnancy [2, 38, 39].

Concerning patients with malignancy, further considerations are required. Women with BRCA mutations could have a decreased ovarian reserve [40]. Stimulation of oocytes in patients with hormone-sensitive cancers is questionable [41]; conventional ovarian stimulation causes supra-physiological estradiol levels, and women with estrogen receptor-positive (ER+) tumors may be unsuitable [41, 42]. In this regard, reliable data suggest that combination protocols with aromatase inhibitors and standard GnRH antagonist stimulation decrease the supra-physiological levels of estrogens [43, 44]. A recent systematic review found that letrozole had no detrimental effect on the disease-free survival period in breast cancer patients. However, there was a lack of good-quality evidence [45].

4.2 Stimulation protocols in fertility preservation

Patients who undergo controlled ovarian stimulation for fertility-preservation are usually responsive to moderate stimulation schedules for gonadotropins [46–48]. In conventional assisted reproductive technology, controlled ovarian stimulation begins at the early or mid-luteal follicular phase and takes 2–4 weeks. Therefore, traditional controlled ovarian stimulation can require up to 2–6 weeks to be completed from the moment of a cancer diagnosis.

Nevertheless, controlled ovarian stimulation therapy can start at any point of the menstrual cycle [49]. This possibility is adopted in the random-start ovarian stimulation protocol, which induces controlled ovarian stimulation immediately and regardless of the menstrual cycle phase. This method is becoming an established method in fertility preservation strategies for cancer patients, enabling oocyte retrieval in most cases with no more than two weeks.

A systematic review (251 patients) comparing ovarian stimulation cycles initiated in the luteal phase and the follicular phase reported that the luteal phase group required longer stimulation days and higher gonadotropin doses [50].

However, the number of retrieved oocytes did not differ, and oocytes obtained in the luteal phase were fertilized more efficiently [50]. Two studies involving 347 cancer patients undergoing ovarian stimulation for fertility preservation compared conventional vs. random-start ovarian stimulation [51, 52]. They reported no significant differences in the number of retrieved oocytes and gonadotropin doses [51, 52].

In a study by Cavagna *et al.* [53], they reported outcomes of random-start protocols in different cycle phases such as early follicular phase ($n = 41$), late follicular phase ($n = 21$), and luteal phase ($n = 47$). They found that the number of retrieved oocytes and the maturity rates were similar, although significantly higher gonadotropin doses were required in cycles initiated in the luteal phase.

An alternative controlled ovarian stimulation protocol is the double stimulation or so-called “dual stimulation” proposed for poor responder patients. It involves two stimulation protocols within the same menstrual cycle [54]. The first protocol is initiated in the follicular phase, then the second protocol begins immediately after the oocyte pick-up, in the luteal phase of the same cycle. Double stimulation provides two oocyte pick-ups that are performed approximately two weeks apart. It allows gathering more oocytes in a shorter period, which is ideal for women with a cancer diagnosis [54]. The current evidence shows that the double stimulation protocol is safe and provides sufficient oocyte quality. A study comparing 100 patients in the dual stimulation group and 197 in the conventional single-cycle stimulation group concluded that the cumulative live birth rate (LBR) (15% vs. 7%) and the rate of euploid blastocysts (31% vs. 14%) are higher in the dual stimulation group [54].

4.3 *In vitro* maturation of oocytes and cryopreservation

In vitro maturation (IVM) of human germinal vesicle (GV)-stage oocytes were defined by Edwards *et al.* [55] in 1969. In 1994, immature oocytes were surgically obtained from patients with polycystic ovary syndrome (PCOS), and IVM was consequently applied. A live birth took place after this treatment [56]. Live births were achieved with frozen-thawed embryos acquired from IVM oocytes of patients who overcame cancer in Singapore in 2014 [57]. This procedure has the advantage to remove the risk of reintroducing malignant cells related to the use of ovarian tissue [58]. Furthermore, IVM of GV-stage oocytes prevents any delay in anticancer treatments, given that no hormonal stimulation is required [59]. IVM is currently used in many centers, with a 20 to 35% live birth rate from cryopreserved IVM oocytes, improved cryopreservation protocols, and culture media [60, 61]. However, there is still not clear evidence comparing the success rate between IVM oocytes and vitrified-warmed mature oocytes [61, 62].

4.4 Embryo cryopreservation

Since the first pregnancy in 1985 [63], embryos' cryopreservation is widely used in assisted reproduction [64]. The technical changeover from slow freezing to vitrification has

led to successful results [65]. However, as compared to other approaches, this technique requires sperm donors and mature oocytes. Accordingly, the method may not be applicable for single women or prepubertal girls. Moreover, similar to other procedures, this technique is limited by the required administration of ovarian stimulation agents for 10–15 days [36].

4.5 Ovarian tissue cryopreservation and reimplantation

Small pieces of frozen-thawed ovarian tissue allowed the ovarian function restoration in oophorectomized animal models, reporting follicular survival and progression to the antral stage [66]. With the progressive developments of tissue cryopreservation techniques and the application in humans, more than 130 live births have been reported worldwide [67] after the first live birth reported by Donnez *et al.* in 2004 [68].

The recommended ovarian tissue retrieval consists of gathering 4–5 slices of the ovarian cortex of about 1 cm per 4–5 mm per 1.0–1.5 cm in size with the laparoscopic approach. The use of extensive electrocoagulation should be refrained since primordial follicles might be damaged [69]. Unilateral oophorectomy can be used to acquire sufficient tissue samples in prepubertal patients due to the lower ovarian tissue volume [2, 70]. Also, an ovary should be left in situ to enable future orthotopic transplantation. One of the fragments can be used for histopathologic analysis to exclude the risk of malignancy.

Initially, sliced ovarian grafts were transplanted heterotopically [66]. However, in the subsequent trials, ovarian tissue was more frequently transplanted in the pelvis [71]. Orthotopic transplantation technique includes the placement of thawed slices into a peritoneal pocket acquired through a peritoneal incision in the ovarian fossa. Another transplantation technique might be performed by placing tissue slices into the peri-medullar area through a limited ovarian cortex incision [72]. Later, sliced ovarian tissues can be fixed by suturing [2].

Heterotopic transplantation is defined as grafting thawed ovarian tissue outside the pelvic cavity in regions such as abdominal subcutaneous tissue or forearm. Heterotopic transplantation is less used but may be considered for patients with severe pelvic adhesions caused by earlier operations or patients who underwent previous pelvic radiation. Oocytes cultivated in controlled ovarian stimulation cycles can easily be accessed through this technique. Live birth rates varied between 23% and 57.5% in studies reporting fertility rates after ovarian tissue transplantation [2, 70, 72–75].

One of the primary concerns regarding ovarian tissue reimplantation is that the ovarian graft functions are expected to last five to ten years based on the patient's age, and type and duration of gonadotoxic treatments [73]. Another drawback of the procedure is the possible immediate follicular failure due to ischemia/reperfusion (I/R) injury [67, 76], which causes the loss of almost 50% of follicles. For this reason, different studies are investigating vascular growth

factors and antioxidants as a treatment to reduce ischemia-reperfusion injury. Moreover, Oktay *et al.* [77] reported that the transplantation of cryopreserved ovarian tissue with a human decellularized extracellular tissue matrix scaffold could reduce the oxidative stress damage. However, regardless of the growing body of evidence, the technique continues to be an experimental method [41]. Noteworthy, there is still an ongoing debate on the efficiency of the vitrification method for freezing ovarian tissue [78].

One of several significant issues in ovarian tissue preservation is the risk of reseeding malignant cells that may cause re-occurrence of primary cancer [41]. A high risk of malignant cell reimplantation has been primarily observed in hematological malignancies [10, 79, 80], such as leukemia, Hodgkin lymphoma, and non-Hodgkin lymphoma [11]. Ovarian metastases have been additionally observed in gastric cancer (55.8%), colon cancer (26.6%), and lung cancer (23.4%) [81]. Moreover, frequent are the ovarian metastasis of gynecological malignancies, such as endometrial cancers, cervical adenocarcinomas, and tubal cancers [82]. In adenocarcinomas of the cervix, the rate of ovarian metastasis is relatively high, with an incidence of 6.8%, while in squamous cell cervical cancer, it is about 0.7–2.5%. However, no ovarian metastasis has been shown in cervical cancer patients with grafted ovarian tissue until now [83]. The risk of ovarian metastasis in the initial phase of endometrial cancer is relatively low, with an incidence of 1.9% [81]. Nevertheless, breast cancer is the most frequent malignancy in reproductive age women, with approximately 55% of the cases occurring in women under 40 years. In these patients, only a few studies assessed the effects of cryopreserved ovarian cortex transplantation techniques [2], and for late-stage breast cancer, the risk of ovarian metastasis is about 13.2–37.8%; therefore, caution should be exercised in these cases.

To overcome this issue, researchers should focus on specific molecular markers or other methods to identify minimal residual disease in grafted tissue [10, 84]. In this regard, some researchers have proposed transplanting the ovarian tissue into immune-deficient mice before implantation to identify the presence of cancer [85]. Moreover, some researchers suggested using the *in vitro* maturation of the oocytes derived from the collected ovarian tissue. The technique consists of the aspiration of immature oocytes from the ovarian tissue samples with subsequent *in vitro* maturation and vitrification [86]. This procedure is considered ideal for cancer patients with transplanted ovarian tissue at high risk of tumor seeding [27, 33]. Hourvitz *et al.* [87] reported data from more than 100 patients who have cancer. According to the report, the success rate of this approach was more than 50%. Published data from more than 40 cancer patients aged between 2 and 18 years reported an oocyte maturation rate of 10–30% [9, 88, 89]. Two live births were reported using *in vitro*-matured oocytes from ovarian tissue [57, 90].

5. Borderline ovarian tumors

Borderline ovarian tumors (BOT) are ovarian malignancies in which maintaining fertility is an option. These tumors have low malignant potential with an overall good prognosis and account for 10–15% of all ovarian epithelial cancers [91–93]. Although most patients diagnosed with BOT need follow-up with little or no chemotherapy, the surgical procedure of reference induces fertility loss or reduce ovarian reserve. Moreover, the delay of pregnancies for 2–5 years is recommended [91]. Therefore, fertility-sparing considerations are required. Regarding controlled ovarian hyperstimulation in patients with BOT, who are candidates for cryopreservation of oocytes, one concern is the possible tumor spillage during the oocyte pick-up. In this regard, preoperative confirmation of BOT is recommended, and some researchers have described that preoperative diagnosis with imaging studies is reliable [92–95]. Controlled ovarian hyperstimulation may also increase BOT progression. Moreover, surgery following a controlled ovarian hyperstimulation treatment may increase ovarian cortical bleeding due to the substantial electrocoagulation for increased vascularity. In these cases, surgery should be postponed until 2–6 weeks after controlled ovarian hyperstimulation for corpora luteal resolution [91, 94–97].

Regarding cryopreservation of ovarian tissue, it can be collected during the surgical treatment of BOT. Fain-Kahn *et al.* [97] stated that cryopreservation of the ovarian cortex should be performed in women with BOT recurrence in one ovary and women with bilateral BOT. However, a case series showed that the macroscopic inspection of affected ovaries was not always useful in identifying the best unaffected ovarian cortex site [97]. Moreover, concerns of tumor seeding are still present [98].

Regarding the effect of fertility-sparing surgery on oncological outcomes in women with BOTs, a study involving 2946 patients reported that fertility-preserving surgery was significantly associated with worse disease-specific survival in patients aged 50 years, but not in younger aged patients [96]. However, there is still a lack of sufficient evidence regarding the safety of fertility-sparing surgery for BOT patients.

6. Conclusions

Fertility preservation methods are becoming more common thanks to the improved effectiveness of oncologic treatments and early diagnosis implementation. More and more females are being affected by iatrogenic ovarian failure and loss of fertility due to these procedures. In this scenario, oocyte and embryo cryopreservation appear feasible, safe, and effective treatment able to preserve fertility in cancer patients of reproductive age. Conversely, cryopreservation of the ovarian tissue may be used for prepubertal girls and patients needing urgent cancer care. The procedure by which aspirated ovarian follicles can be matured *in vitro* and then used is still experimental and still needs more evidence on

its effectiveness. Indeed, different steps are yet required to improve the effectiveness of available fertility preservation options and develop new fertility-sparing strategies for young cancer patients, such as developing substances for ovarian protection, improving ovarian grafting and post-implantation follicular survival, reducing the risk of reseeding malignancies with ovarian biopsies, developing more effective *in vitro* maturation system for primordial follicles, implementing the aspiration of immature oocytes from pre-antral and small antral ovarian follicles independently from cycle phase, and investigating new medications able to limit the ischemia/reperfusion injury.

Author contributions

SK, CK, SDS, MM and MT designed the research study. SK, CK and SDS performed the research. MT, BA and AA provided help and advice on the writing. SK and CK analyzed the data. SK and CK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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