Review

Preservation of Fertility in Females Treated for Cancer

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Abstract

Advancements of diagnosis and treatment have substantially improved cancer survival rates in the last few decades. The increasing number of survivors focuses attention on long-term effects caused by cancer treatment and its impact on quality of life. Ovarian failure is one of the major sequelae of cytotoxic chemotherapy and/or radiotherapy in female children and reproductive-age women. Oncologists should address the patients about fertility preservation options before therapy. Embryo cryopreservation is the only well-established method for females in preserving fertility; however other strategies including ovarian suppression, ovarian transposition and cryopreservation of oocytes and ovarian tissue are still experimental. Patients need advice and to know which are the most practical options for them. This article reviews the available fertility preservation methods in women, and the related issues including normal physiology of the ovary, effect of anticancer therapy on fertility, role of the oncologist and ethics. We performed a MEDLINE search from 1971 to 2011 in a similar way as Jensen *et al.* 2011, using the following MeSH terms: antineoplastic agents; ovarian failure; premature; infertility, female; fertility preservation; child and cancer; reproductive technologies, assisted.

Key words: Fertility Preservation, Cancer patient, Females

Introduction

Over the past two decades, cancer incidence rates continue to increase [1]. Among women, under the age of 40, approximately 1 in 47 will develop cancer [2]. Due to advancements in cancer therapy, the overall death rate decreased by 1.5% per year in females from 2002 through 2007 and 0.8% per year from 1994 through 2002 [2]. Between 1990/1991 and 2007, cancer death rates decreased by 13.9% in women, the reduction in death rates for breast, colorectal, ovarian and cervical cancers and non-Hodgkin lymphoma and leukemia accounted for almost 75% of the decrease [2]. However, the treatments of cancer often cause infertility or premature gonadal failure [3-5]. An increasing proportion of reproductive-age women who undergo anticancer therapy have concerns about their future fertility and parenthood [6].

Clinical infertility is defined as the inability to conceive after one year or more of intercourse without contraception during the fertile phase of the menstrual cycle [7]. The baseline incidence of sterility is estimated at 8% for women aged 19 to 26 years, and 18% for women aged 35 to 39 years [7]. Risks of permanent infertility and compromised fertility after cancer treatment vary and depend on the type of cancer, the major treatments, age of the patient, the time available before treatments, and the risk of ovarian metastasis [8]. Current techniques of fertility preservation, such as cryopreservation of embryos, oocytes and ovaries, ovarian transposition, ovarian suppression, conservative surgery and gonadal

shielding are often available in female cancer patients [9]. Fertility preservation should be considered as early during treatment plan as possible [9]. Oncologists should inform young patients about these options in a comprehensive pattern with focus on medical and ethical issues [9].

Normal physiology of the ovary

Oogenesis is a limited event which ceases during fetal development with a finite number of oocytes present at birth [7,10,11]. At about 20 weeks in utero, a peak in a number of oogonia approximately 6–7 million is seen in a female infant's ovaries. This number is reduced after follicular assembly. Some of the germ cells do not get into follicular structures and are lost [12,13]. There is a abruptly drops in this number by birth to about 1–2 million, at puberty only about 200,000–500,000 oocytes remain, but at menopause less than 1000 remain [14-19]. Only a few oocytes will be mature and undergo ovulation during reproductive years [20]. The majority of oocytes will be lost due to the atresia [20], which is a inherent process until menopause in spite of pregnancy, amenorrhea or contraceptive use [10], however mediated by aging, disease mechanism like premature ovarian failure, cancer, or iatrogenic causes [12]. In the prepubescent period, primordial germ cells generate oogonia, these cells in turn undergo mitosis to produce oocytes, which will be arrested in meiosis I as primordial follicles until puberty [20]. Once puberty ensues, meiosis is resumed, a monthly cohort of follicles is selected to grow and develop, but only one of these oocytes will complete meiosis I and be arrested in metaphase II until fertilization [21,22].

Recent studies suggested that primordial germ cells *in vitro* are capable of forming oogonia, some species of mammals a type of ovarian stem cell may persist throughout adulthood, and ovarian regeneration may occur from stem cells in the bone marrow [23-25]. Since all the findings are performed in animal models and have not yet been replicated in other laboratories, the fertility potential of stem cells in humans remains answered [26]. At present there is no evidence that primordial germ cells that can produce new oocytes are present in ovaries of women. [27].

Effect of anticancer therapy/cancer on fertility

Multi-agent chemotherapy, radiotherapy, ovarian metastasis and surgical removal of the adnexa could result in premature ovarian failure, which is attributed to decreased ovarian reserve [10]. Ovarian reserve reflects the number of oocytes in a woman's ovaries, and is highly associated with fertility [18].

The side effect of therapies depend on the type of cancer, drug, method of administration, size/location of the irradiation field, dose/dose-intensity, age, and the prescribed treatment [15].

Chemotherapy

Most multi-chemotherapeutic agents can affect oocytes, granulosa and theca cells, it will be detrimental to ovarian reserve and induce amenorrhea which may often be reversible [28]. The alkylating agents (cyclophosphamide, procarbazine, ifosfamide, nitrosoureas, chlorambucil, melphalan, busulfan) are more likely to pose this risk to gametes, because they are non-cell-cycle specific and could cause damage even to "resting" oocytes [29-34]. Cyclophosphamide is the most commonly implicated agent in causing damage to ovaries, particularly to the primordial follicles [35]. The odds ratio for inducing premature ovarian failure with cyclophosphamide exposure were reported between 4-9.3 compared to those in unexposed patients [35]. Kenney *et al.* [36] reported that cyclophosphamide caused follicular damage in a dose-dependent manner, however, Meirow *et al.* [37] described the destructions of primordial follicles were observed at all levels of cyclophosphamide exposure, even at low doses of 20 mg/kg. Cyclophosphamide and related combination routinely used for breast cancer and hematopoietic stem cell transplantation (HSCT) [38]. Platinum-based compounds and taxanes are also often applied in breast cancer, cervical cancer and germ cell tumors–all common in premenopausal women [38]. Age plays an important role in the risk of chemotherapy-induced ovarian failure. Older women (with low primordial follicle pool) appear more susceptible to permanent ovarian damage compared with young women (with higher primordial follicle number) [39]. This explains why the incidence of ovarian failure after chemotherapy in breast cancer patients is more than 50% in age over 40 compared to 30% in those less than age 35 [40].

Radiotherapy

Radiotherapy may affected fertility not only by direct total body, low abdominal, pelvic, or spinal radiation, but also scattered radiation which can cause gonadal failure even if ovaries are outside of the radiation field [5,6]. Human oocyte is extremely sensitive to ionizing radiation, direct radiation cause a dose and age-related reduction in the ovarian follicular pool, dose of less than 2 Gy is enough to destroy half of the oocyte population, dose of more than 6 Gy usually causes irreversible ovarian failure [5,6]. Compared with younger women, older patients are at higher risk for developing infertility after radiother-

apy due to the low primordial follicle pool and the high radiation-induced chromosome damage in the oocytes [41]. It appears to be more toxic when ionizing radiation is given in a single dose compared to fractionated doses [9]. Anatomic or vascular changes to the uterus, cervix or vagina and interferes with the hormonal balance from ionizing radiation may also affected fertility [42,43]. In addition, radiotherapy has a potential deleterious effects on the uterus (as consequence of uterine vasculature damage, endometrium damage and myometrial fibrosis) and the hypothalamic-pituitary axis (as consequence of cranial irradiation in central nervous tumours or total body irradiation in hematological malignancies) [42,43].

Metastasis and surgery

It is logical that there is a risk of ovarian metastasis if the patient survives malignant tumor. Hematologic diseases such as leukaemias and Burkitt's lymphoma are high risk in ovarian involvement, some advanced stage solid tumours such as breast and colon cancers are moderate risk [5,6]. Most other cancers in reproductive age women have low risk of ovarian metastasis, including breast cancer in stage I-III, squamous cell carcinoma of the cervix, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, osteogenic sarcoma, Ewing's sarcoma, etc. [6] However, in spite of the risk of metastatic disease, histopathological or molecular biological analysis should be performed to determine the presence of cancer cells in the ovarian tissue before and after cryopreservation of ovaries [6].

Gynecologic surgery is the major therapy for gynecologic malignancies, the radical operations remove the key fertility organs (cervix, uterus, fallopian tubes and ovaries) and cause permanent damage to fertility and endocrine function [10]. The current trend of treatment is the conservative surgery with the intent of maintain organs as much as possible for reproductive function. It has been suggested in the stage 1A2-IB cervical cancers, the recurrence rates of radical trachelectomy have no significant higher than that of radical hysterectomy [44]. Studies also showed in the ovarian germ cell tumors, conservative surgery will not significant affect the risk of recurrence compared with historical controls [45,46]. However, the view of fertility-sparing surgery has not received wide acceptance, for these trials are often limited in size and lack randomized controls [9].

Fertility preservation options in females

Each individual has her own situation; this section reviewed the currently available fertility preser-

vation options in female and the relevant clinical applied scope (Figure 1A and B). Before cancer treatments, if the circumstances permit, women can be treated with *in vitro* fertilization to create embryos for cryopreservation or with surgically moving ovaries out of the radiation field [9,13]. Embryo cryopreservation is the only well-established technique used for fertility preservation in female, however, it may not be appropriate for every patient due to age, delay in therapy, type and stage of cancer, and her partner [13]. Most other options such as cryopreservation of oocyte and ovarian tissue, and ovarian suppression remain in the area of research [5]. Conservative surgical techniques (preserving ovaries in early ovarian tumors, preserving uterus in early cervical cancers) and gonadal shielding are also available approaches but will not be further discussed in this paper.

Ovarian suppression

Clinical data support a viewpoint that the prepubertal girls have greater tolerance than postpubertal women during gonadotoxic treatments [47]. Ovarian suppression with gonadotropin-releasing hormone agonist (GnRH-a) during chemotherapy has been advocated by some oncologists [47]. One hypothesis is that the administration of GnRH-a could make the ovary an endocrine prepubertal state with less susceptible to cancer treatments [47]. Studies in monkeys successful demonstrated the protective role of GnRH-a against the primordial follicle loss associated with cyclophosphamide, however failed to found the same role in ionizing radiation-induced ovarian injuries [48,49]. The available clinical researches are highly controversial, some meta-analysis showed a benefit of ovarian suppression by GnRH-a, but other studies did not show a benefit of this approach [50,51]. Oncologists discussed that there was little possible for GnRH-a to preserve ovarian reserve due to the total 90% of follicle pool in an adult was primordial follicles which recruited through a follicle stimulating hormone (FSH) independent manner [9,52]. There was a theoretical higher risk of hormone receptor-positive cancers recurrence, including breast, endometrial and ovarian cancer [9,52]. The American Society of Clinical Oncology concludes there is insufficient evidence that ovarian suppression protects fertility from gonadotoxic therapies [9]. However, GnRH-a administration has been used widely due to the readily available [9]. A large randomized clinical study with long-term follow-up should be performed to clear the safety and efficacy of GnRH-a treatment.
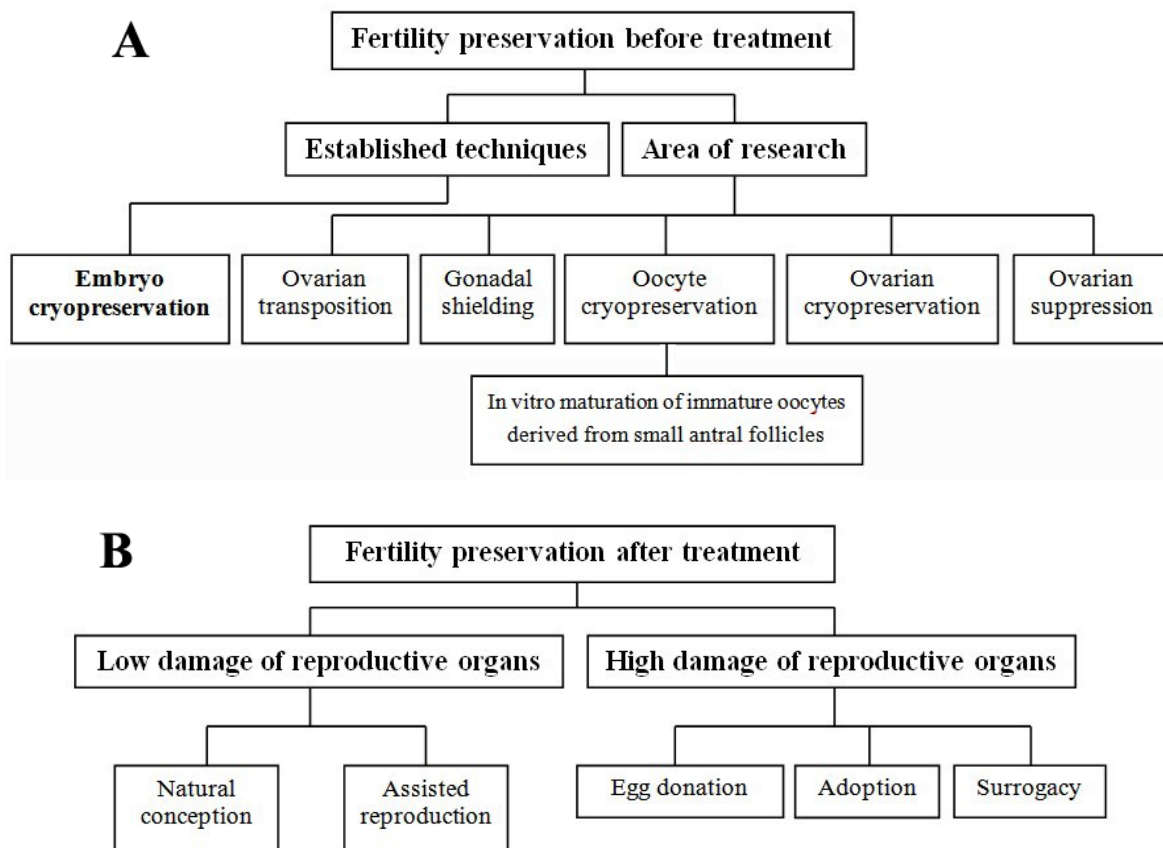
Figure 1. Summary of fertility preservation strategies in female cancer patients before (A) and after (B) treatment.

Ovarian transposition

A standard radiation dose often causes premature ovarian failure in the treatment of genital, intestinal or urinary tract tumors [10]. Ovarian transposition (oophoropexy) can be used in the pelvic radiotherapy through surgically moving the ovaries above the pelvic brim and out of the planned exposure field to avoid radiation injuries [9,10]. This procedure should be done as close as possible to the radiotherapy for the possibility of migration of the ovaries [10]. If laparotomy is needed for tumor removal, oophoropexy can be performed simultaneously, or if it not need, this operation can be accomplished laparoscopically [53-55]. The success with resumption of menstruation by oophoropexy before radiation varies between 16 and 90% [53-55]. This variation is mainly affected by scatter radiation which is approximately 8-15% of the radiation dose in pelvic region, and by ovarian vascular change caused by surgical procedure in separation of utero ovarian ligament and mesovarium [56]. Spontaneous pregnancy is generally not happen when ovaries were transposed out of their original sites [9]. Ovarian repositioning or trans-

abdominal oocyte retrieval may often be needed to restore fertility [9]. If the radiotherapy is whole-body radiation or it combines with chemotherapy, the strategy will not work [9,10]. As a result, the patients should be selected carefully before oophoropexy to maximize their benefits.

Cryopreservation options

Embryos Embryo cryopreservation is used routinely for storage of surplus embryos during *in vitro* fertilization [9,10]. This procedure involves 2-6 weeks of ovarian stimulation [daily injections of follicle stimulating hormone (FSH) from the onset of menses, to collect mature oocytes with an injection of human chorionic gonadotropin (GSH) to start ovulation], *in vitro* fertilization (a partner or donor sperm is required) and cryopreservation [57]. The delay of 2-6 weeks in therapy initiation may make it not applicable for women with highly aggressive malignancies, such as leukemia and sarcomas, which require immediate treatment [58]. It might also be noted that ovarian stimulation causes high serum concentrations of estrogen in some hormone-sensitive tumors [59]. For these patients, alternative protocols have been inves-

tigated to reduce the potential risk of estrogen exposure, such as the use of tamoxifen in breast cancer and the use of letrozole in endometrial cancer, and showed some individual successes [6]. Despite these limitations, reported survival rates per thawed embryo range from 35-90%, implantation rates from 8-30%, and cumulative pregnancy rates of 30-40% [60-62]. Although the pregnancy rates are encouraging, this technique may be impossible because of lack of partner and the patient not wanting a sperm donor [6,9].

Oocytes Oocytes cryopreservation (unfertilized) is an alternative option for fertility preservation in patients who do not wish to use donor sperm [6]. The process requires ovarian stimulation, therefore, it has similar limitations as embryo cryopreservation, including impossibility for girls before puberty, delay in therapy and high hormonal levels exposure [6]. The oocytes can be aspirated in the operating theatre or directly from ovaries [6]. Unfertilized oocytes are more sensitive to cryodamage than embryos due to their large size, water content, high-lipid content and temperature-sensitive spindle [63]. The overall pregnancy rate per cryopreserved oocyte is only about 2%, which is lower than the rate using embryo cryopreservation and much lower than the rate using fresh oocytes [64-66]. Studies showed the improvement of cryopreservation could increase success rates, there is success in oocyte freezing by vitrification but slow freezing is also used [67,68]. Compared with slow freezing, vitrification is a more efficient method for freezing the oocytes produced from *in vitro* maturation [67,68]. Collection of immature oocytes with *in vitro* maturation and subsequent cryopreservation could be done with mild ovarian stimulation, thus avoiding the delay in cancer treatment and high hormonal levels exposure [69,70]. Immature oocytes seem more resistant to cryodamage due to their lower cell volume and lack of metaphase spindle, however the inefficiency of *in vitro* maturation also results in low success rates [71]. At present, oocyte cryopreservation should only be operated in the centers with necessary expertises, a much higher success rate must be obtained so that this option can be widely offered to females [9].

Ovarian tissue Ovarian tissue cryopreservation is an experimental technique, but it precludes the ovarian stimulation and sperm donor in embryo/oocyte cryopreservation [5,6]. It may be the only acceptable method that can be offered to prepubertal patients, and a preferred method for women who cannot delay their therapies for the hormonal stimulation to harvest oocytes and/or create embryos [8]. Generally, ovarian cortical tissue is removed by a laparoscope followed by subsequent cryopreservation, ovarian cortex (contains primordial follicles) can be cryopreserved with great efficiency [9]. After treatment, ovarian tissue is thawed and reimplanted orthotopically in the ovarian fossa or heterotopically in the forearm or abdominal wall [10]. Although studies are case reports, there have been successful live births after orthotopic transplantation in cancer patients, to date, human livebirths have only been achieved after grafting to the ovary [72]. Same as a common transplantation, the interval between transplant and revascularization will cause ischemia-reperfusion injury after ovarian transplantation [10]. Most primordial follicles could survive the freeze-thaw cycle, however, nearly a two-thirds or more of these follicles are lost after transplantation [13]. Recent studies suggest that host treatment with melatonin or graft incubation with HA-rich biological glue, especially when combined with VEGF-A and vitamin E improves graft survival [73,74]. The main concern with re-implanted tissue is the theoretical risk of ovarian contamination in some malignancies [75-78]. There is more and more evidence of possible reseeding of cancer as malignant cells might exist in the ovary, especially in lymphomas and leukemia [75-78]. In the future, *in vitro* culture of primordial follicles may eliminate this risk, at present it had been successful only in the mouse but small antral follicles have been obtained in humans [79,80].

Role of the oncologist

Infertility is often low understood by patients, oncologists have a responsibility to inform them that the cancer therapy may permanently affect their fertility [9]. Fertility preservation has positive influences on the mental health of a patient as it is a way of saying there is normal life after cancer [5,6]. There is no evidence that currently used fertility preservation options decrease the success of cancer therapy, adversely affect a survivor's health, increase the risk of maternal or perinatal complications, or compromise the health of subsequent children [9,81,82]. Oncologists must pay attention to the plans for fertility preservation as medical necessity.

Some fertility preservation methods in women need timing because of menstruation [9], if gonadal toxicity is unavoidable, oncologists should refer the candidates to fertility specialists as soon as possible, so that avoid missing opportunities. Fertility specialists will help females to perform cryopreservation of oocytes, embryos or ovarian tissue, and provide other assisted reproductive technologies [83]. The most used methods for cryopreservation are vitrification and slow freezing, in the process, cryoprotectant, such

as dimethyl sulfoxide, polyethylene glycol and glycerine etc., is needed to prevent frozen injury [84]. A psychologist should be available to help a woman has moderate to severe distress about potential infertility, since structured and cognitive behavioral counseling can reduce anxiety and depression [85-87].

Ethics

Harvesting ovarian tissues will raise a wide range of ethical issues which include the available assisted techniques, safety of the tissue harvesting, subsequent use, possible implications for the progeny, as well as some cases that might be posed by special religious beliefs or cultural values that preclude assisted reproductive techniques [70]. When treating cancer survivors, the most ethical consideration is whether assisted reproduction techniques pose risks to the potential children [88], especially whether offsprings are themselves at increased risk of cancer (a hereditary cancer risk should not be regarded). Some large registry studies give us encouragement; they revealed there is no clear increased risk to cancers, genetic abnormalities, or birth defects in the children of cancer survivors [9]. There are some ethicists suggested it unethical to enable reproduction for cancer survivors whose lifetime may be reduced by diseases, for it unfairly makes a child bereft of a parent [88]. However, the Ethics Committee of the American Society for Reproductive Medicine concludes the concern is an insufficient argument to deny cancer patients assistance in reproducing [88]. Quite different from fertility preservation in adult females, there are special ethical issues in pediatric and adolescent patients [89]. Fertility preservation in a minor is only allowed: firstly, when appropriate permission of a parent and assent of a child with the ability to understand are both obtained; secondly, assisted procedures are justified by the anticipated benefit which is at least as favorable as that provided by alternatives [89]. Regardless of whether the patient lives or dies, the disposition of gametes must be considered [88]. Oocytes and ovarian tissue are the sole possessions of a person from whom they were removed, however, an embryo could belong to both parties, and the embryo should not be used for reproduction unless they both agree [90]. If the patient dies, an extreme controversial issue is the use of her gametes, and this issue is acceptable only if the decision is made by the surviving partner and the written permission given by the deceased [91].

Conclusions

Unlike sperm production may recover, the loss of oocytes is permanent [92]. Fertility preservation is often available but ignored in the female undergoing cancer treatment [5,9]. Embryo cryopreservation is considered standard practice, other methods such as ovarian suppression, ovarian transposition and cryopreservation of oocytes and ovarian tissue should be considered investigational, they could only be performed in specific centers [6,9]. The fertility preservation approaches vary depending on the patient's age, the type of cancer, the time available and the risk of ovarian metastasis [6]. At present, the primary purpose of medical intervention on malignancy is to cure the diseases, however, the generally trend is that oncologists should pay attention to the damage of reproductive system and consider fertility preservation options to help the patients preserve fertility during treatment.

Acknowledgments

Competing Interests

The authors have declared that no competing interest exists.

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