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REVIEW ARTICLE

Restoring Fertility in Cancer Survivors: Ovarian Tissue Cryopreservation or Assisted Reproduction Technique

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ABSTRACT

Fertility restoration in cancer patients is gaining a very important role in the field of reproductive medicine, due to the rising incidence of cancer as well as its early detection and improved survival rate. The two options for achieving this aim are ovarian tissue cryopreservation (OTC) and assisted reproductive treatment (ART) through oocyte or embryo cryopreservation after IVF. However, both these have some advantages and disadvantages over each other. OTC is still in experimental phase but is growing faster as an important part of fertility restoration. ART is time tested method which can be relied upon to a great extent, but there are some situations where ART cannot meet the expectations. This review is an overview of the pros and cons of both these options and the status of these methods in the present scenario of fertility preservation.

Keywords: Embryo cryopreservation, Oocyte cryopreservation, Ovarian tissue cryopreservation.

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INTRODUCTION

Diagnosis of cancer is a crucial event in the life of any person. Its impact depends on the type of cancer, available treatment, and the physical, mental, and social resources of the person. Younger persons have the additional risk of loss of reproductive function and the opportunity to have offspring. A common long-term effect of cancer therapy may be decreased fertility potential. Due to drastic increase in survival rates, there is a growing demand for different fertility restoring options. Most of the cancer patients can now dream of starting a family of their own. Assisted reproductive technology currently offers a variety of fertility preservation techniques.

The discussion of fertility preservation should be done prior to starting treatment; however, due to unawareness, this aspect of treatment is generally neglected by the patients as well as health professionals. Numerous techniques are available for fertility preservation that can be used individually or together in the same patient to maximize efficiency. Ovarian tissue cryopreservation (OTC) is experimental in present scenario, but it presents a potential for wider clinical application and the advantage of opening the fertility window for a longer duration. Oocyte and embryo cryopreservation are now established techniques but have their limitations.

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EFFECTS OF CANCER TREATMENT ON REPRODUCTIVE ORGANS

Both chemotherapy and radiotherapy have a major impact on reproductive potential and fertility preservation procedures should be carried out prior to these treatments.

Ovarian Effects

Chemotherapy causes deoxyribonucleic acid abnormalities as well as oxidative damage in somatic as well as germ cells,[1] which may lead to apoptotic death in oocytes, also referred to as the burnout effect. Aneuploidy and early miscarriages can be caused by genetic effects on germ cells.

Impact of chemotherapeutic drugs on the ovary depends on the type of the chemotherapeutic agent used, dose given, age of the patient, and her baseline ovarian reserve. In some cases, it can cause complete ovarian atrophy also. Older women are at a higher risk of premature ovarian failure (POF) due to lower ovarian reserve.[2]

Due to effect of chemotherapy on mature oocytes, an increase in congenital malformations is seen when conception occurs within 3 months of treatment. It is advisable to delay the conception by 6 months because mutagenesis is maximum during maturation phase of

oocyte. However, the exact time interval between the treatment and oocyte collection for preservation is not well defined.[3-5]

Radiotherapy also has a very damaging effect on ovary and depends on the age of the patient and dose of the ovarian exposure. In general, <2 Gy is the median lethal dose (LD50) of radiation to oocyte. Effective sterilizing dose (ESD) is the dose of fractionated radiotherapy (Gy) at which POF occurs immediately after treatment in 97.5% of patients. Only 6 Gy is sufficient to cause permanent ovarian failure in women over 40. The number of primordial follicles present at the time of treatment and the dose of radiation received by the ovaries determines the fertility "window." Ovarian failure has been reported in 90% of patients following total body irradiation (TBI) (10–15.75 Gy) and in females treated with total abdominal irradiation (20–30 Gy) during childhood, the percentage increases to 97%.[6]

Uterine Effects

Exposure to radiation can lead to decreased vascularity, myometrial hypoxia leading to fibrosis and hormone-dependent endometrial inadequacy, which can adversely affect the reproductive outcomes subsequently. The uterine volume is lower and endometrium atrophies completely if there is direct radiation.

Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation involves retrieving ovarian cortical tissue which has abundant primordial follicles, prior to ovarian failure. It can be done by either laparoscopy or laparotomy.[7] Ovarian tissue obtained is dissected into small pieces, and then cryopreserved by either slow cooling technique or vitrification. Removal of ovarian stroma before slicing the cortex is considered to be important to reduce the thickness of the tissue. Presence of stromal tissue may impair the permeation of the cryoprotectant into the ovarian cortex, which may result in reduced survival rates of the follicles.[8-10] The thickness of the slices is kept optimal to facilitate equilibration of the cryoprotectant. Although most of the published reports kept the thickness of the slices at 1 mm, the surface area varied from 2 × 2 to 5 × 5 mm.[8-13]

Slow Freezing *vs* Vitrification

The pregnancies and live births reported from human ovarian cryopreservation and transplantation have resulted from slow freezing of ovarian tissue. In a study by Gook et al,[8] rapid freezing of ovarian tissue resulted in a lower proportion of intact oocytes and a higher proportion of vacuolated oocytes. Vitrification also leads to an increase in necrosis in human ovarian tissue.[14] However, a novel technique of needle immersion vitrification has been reported recently to yield satisfactory survival of follicles in both human and murine ovarian tissue.[15] In a study by Klocke et al,[16] slow freezing and vitrification resulted in similar morphological integrity after cryopreservation, a similar estradiol release in culture and similar rates of follicular proliferation and apoptosis after culture. According to Zhou et al,[17] vitrification and slow freezing was found to produce similar results in a meta-analysis with respect to intact primordial follicles for the cryopreservation of human ovarian tissue. Factors that can affect the outcome of cryopreservation comprise cryoprotectant, size of tissue fragments, and speed of cooling, and recent studies have proved that vitrification can produce equivalent or better results than slow cooling in preserving ovarian tissue.[15-17]

The ovary has hundreds of primordial follicles that contain immature oocytes. These oocytes are smaller than the mature oocytes, dormant in nature, less differentiated, and do not contain zona.[18] Such immature oocytes could tolerate cryopreservation due to the absence of zona and cortical granules.[19] Ovarian tissue can be obtained from a female cancer patient regardless of age with no delay in cancer therapy. Amorim et al[20] have shown that steroidogenic and gametogenic functions are well preserved in cryopreserved ovarian tissue.

METHODS OF TRANSPLANTATION

Transplantation of ovarian tissue is done after completion of cancer therapy.[7] Transplantation can be orthotopic (into the pelvis) or heterotopic (outside the pelvis, in abdominal wall or forearm).

Orthotopic Transplantation

Orthotopic transplantation involves transplantation of very thin, <1 to 1.5 mm strips of ovarian tissue into either medulla of ovarian remnant or peritoneum of ovarian fossa. Spontaneous pregnancies can occur after orthotopic pelvic transplant and it also provides a favorable environment for follicular development.[21] However, there might be a decrease in the number of primordial follicles. Ovarian reserve tests posttransplantation have reported return of follicle-stimulating hormone (FSH) to basal level, although anti-mullerian hormone levels remain still low, supporting the notion of reduced number of follicles. The number of fragments which can be transplanted is limited and depends on the ovarian size. Also, there is involvement of a surgical procedure for autotransplantation. Many successful pregnancies have been achieved through orthotopic transplantation. Rodriguez-Wallberg et al[22] reported the first successful delivery after transplantation of cryopreserved ovarian cortical tissue

and subsequent *in vitro* fertilization (IVF) in a patient of Ewing's sarcoma. Patient had received sterilizing pelvic radiotherapy (54 Gy) and 40 weeks intensive high-dose chemotherapy for the treatment of Ewing's sarcoma 14 years earlier. However, to obtain fully functional follicular development, repeated transplantation procedures were required. Growth of the transplants as well as the increase in the size of uterus was demonstrated over time on sequential ultrasonographic exams.

Heterotopic Transplantation

Heterotopic transplantation can be done in abdominal wall, forearm or chest wall. This helps in placing the tissue at a location where follicular growth can be easily monitored and oocyte retrieval can be trouble-free. Furthermore, it becomes simpler to keep an eye on cancer recurrence. However, the viability of grafts is compromised due to limited neovascularization and IVF is always required to achieve conception in a heterotopic transplantation. The first ongoing pregnancy from a heterotopic implantation of ovarian tissue has been reported recently by Stern et al[23] from Melbourne, in a patient who had both ovaries removed because of ovarian cancer. The ovarian tissue was transplanted heterotopically into the anterior abdominal wall. Subsequently, IVF was done, two oocytes were recovered after mild stimulation, and embryos implanted into the uterus.

In women over 40 years, follicular survival rate after ovarian transplantation of frozen ovarian tissue is low, thus precluding its use in older women. In younger patients, the amount of ovarian tissue cryopreserved should be proportional to the risk of age-related diminished follicular reserve. Based on the current evidence, removal of both ovaries for cryopreservation is not justified at this time unless the chemotherapy regimen has an extremely high likelihood of inducing complete ovarian failure. There is a fair chance of resuming ovarian function after chemotherapy.[24]

ADVANTAGES OF OVARIAN TISSUE CRYOPRESERVATION

Ovarian tissue cryopreservation has some advantages over oocyte and embryo cryopreservation. There is no delay in starting cancer therapy and it also avoids the risk of ovarian stimulation. Partner or donor sperm is also not needed. It leads to preservation of a larger pool of follicles and ovarian function can be resumed. Ovarian function generally resumes between 60 and 240 days posttransplant and can last for up to 7 years.[24]

In prepubertal girls, OTC is the only option available for preservation of fertility.[25-27] The number of immature oocytes in the ovarian cortex at this age is high and primordial follicles contain prophase I oocytes. Thus, theoretically the chance of restoring fertility is higher, because ovarian cortex contains an increased number of primordial and primary follicles in younger women.[26,28]

Childhood cancer survival rates have increased drastically over the past five decades. Five-year survival rate has reached up to 80%. About 70% of the patients will be alive and cured of their original cancer 10 years after diagnosis.[29]

Cancer treatments are known to cause ovarian dysfunction in children. The increasing life expectancy of children with cancer has created an increasing population of girls and young women at risk of developing premature ovarian insufficiency. Most of the children treated for cancer will sustain a window of opportunity for fertility. However, patients at high risk of premature ovarian insufficiency have limited options for fertility preservation and these methods are still experimental. No live births have been reported till now in females whose ovarian tissue was cryopreserved before puberty.

Transplantations of tissue harvested from prepubertal girls have not yet been reported in humans, but the procedure holds great future prospects. The lowest age at which this approach is recommended is not defined. It is recommended based on multidisciplinary discussions that OTC should be offered from 3 years of age.[27] Moreover, oocytes could be aspirated, matured, and cryopreserved even in premenarchal patients. Evidence from case series and reviews have suggested that the collection of ovarian tissue for freezing by laparoscopy under a general anesthetic is safe and feasible in prepubertal girls as well as in adult women.[30,31]

The authors of two case reports have described the replacement of ovarian tissue in adolescents for the purpose of estrogen production for induction of puberty.[32,33] The evidence of successful hormone production by the ovarian autografts in those reports does suggest that follicular development is possible, potentially allowing for oocyte maturation, ovulation, and fertility; however, the data are not optimal.

Reimplantation of ovarian tissue has resulted in successful pregnancies in some adult women, confirming the potential of this approach.[34] Till now, pregnancy has not been reported after OTC in childhood or adolescence. Majority of girls and young women treated for cancer will have a window of opportunity for fertility restoration in the future once they have survived their original cancer. It is mainly dependent on the nature of the patients' planned treatment and their response.[25] The identification of patients who are at the highest risk of loss of fertility is crucial for justifying the use of an invasive experimental approach.

Selection Criteria

Edinburgh selection criteria are used to predict the patients with cancer who are more likely to develop premature ovarian insufficiency and are therefore, likely to benefit from OTC. As the procedure is invasive, and the success rate in terms of future live births remains unknown, it is necessary to limit OTC to those patients at high risk of premature ovarian insufficiency.[35]

The Edinburgh Selection Criteria

- Age younger than 35 years
- No previous chemotherapy or radiotherapy if 15 years or older at diagnosis, but mild, nongonadotoxic chemotherapy acceptable if younger than 15 years
- A realistic chance of surviving for 5 years
- A high risk of premature ovarian insufficiency (>50%)
- Informed consent (from parents and, where possible, patient)
- Negative serology results for human immunodeficiency virus, syphilis, and hepatitis B
- Not pregnant and no existing children

Ovary Cryopreservation

Cryopreservation of the whole ovary can be regarded as an alternative to ovarian cortical slices as these slices suffer from loss of follicles mainly from ischemic damage and sometimes due to cryoinjury.[36,37] Furthermore, it is a challenge to cryopreserve the whole organ due to its size and involvement of large quantity of tissue and various biomolecules. It also requires expertise in anastomoses of small vessels. High postthaw survival of follicles and well-preserved stromal and vascular tissue has been shown in intact human ovary when cryopreserved with pedicle.[38,39] These papers demonstrated successful perfusion of cryoprotectant through the ovarian artery followed by slow cooling. Although these advances are promising, cryopreservation of the intact ovary has a long way to go before its application to patients for an effective treatment. Also, if the whole ovary gets ischemic, there is no other way left for restoration of fertility.

DISADVANTAGES OF OVARIAN TISSUE CRYOPRESERVATION

Despite the luring benefits, there are few major concerns with cryopreservation and transplantation of ovarian tissue. Firstly, the procedure is invasive and carries an unacceptable operative risk in immune compromised and pancytopenic children with cancer who might be at increased risk of bleeding and infection.

The second major concern is regarding reseeding tumor cells following ovarian tissue transplantation, especially for malignancies like leukemias that are systemic in nature.

Autologous transplantation is contraindicated in situations where cancer cells may be present in the cryopreserved ovarian tissue. The reliability of screening with histological evaluation or with tumor markers is not well-defined and there is no certainty regarding risk reduction of reseeding tumor cells by carrying out these tests.[40]

Patients harboring the Breast cancer gene1 (BRCA1) or BRCA2 gene may also be at risk. In patients at risk of development of ovarian cancer, a temporary heterotopic transplantation followed by removal of the tissue after childbearing can be an option.[41] It is better to avoid OTC for transplantation in patients carrying a BRCA mutation due to the increased risk of ovarian cancer in this population. Any epithelial ovarian malignancy in early stage can be easily missed, as cryopreservation of ovarian tissue prevents detailed pathologic examination of the ovaries.

Oocyte or Embryo Cryopreservation

In patients with cancer, the other option to restore fertility is assisted reproduction techniques (ART) where patients undergo controlled ovarian stimulation with IVF or intracytoplasmic sperm injection (ICSI). The collected oocytes are fertilized with their partner's sperm and the resulting embryos are frozen. These embryos are used for transfer into the patients after their cancer is cured.

Quintero et al[42] compared IVF cycle data among 32 women who underwent IVF prior to cancer treatment with 21 age-matched male factor or tubal factor infertility IVF controls. No difference was reported in the total amount of medication used for ovarian stimulation and number of oocytes retrieved between the two groups.[42] In a subsequent study, these authors found no significant differences in terms of number of oocytes retrieved and the number of oocytes fertilized between 50 women with cancer and 50 age-matched controls; however, cancer patients required longer stimulation and greater amounts of medication.[43] In one study by Knopman et al,[44] no significant difference was found with regard to FSH, peak estradiol, number of eggs retrieved among 27 women with breast, uterine, ovarian cancer, Hodgkin's lymphoma, and age-matched control group.

Similarly, Robertson et al[45] found no difference in the number of oocytes obtained or embryos created comparing women with cancer and age-matched control patients, albeit a significantly lower peak estradiol in the cancer patients was reported. Furthermore, there were no significant differences in women with localized cancers *vs* women with systemic disease.

In contrast, Klock et al[46] reported significant differences in peak estradiol, number of oocytes retrieved, and cancellation rates between cancer patients and age-matched control patients, but no difference in the

number of zygotes created. In addition, Domingo et al[47] observed that women with hormonally sensitive cancers have decreased ovarian response to controlled ovarian hyperstimulation (COH) even before treatment and thus, number of oocytes retrieved is lesser when compared to noncancer patients.

Based on these studies it appears that many women with cancer have sufficient response to COH to undergo a successful oocyte harvest, although not necessarily equivalent to age-matched patients undergoing IVF for infertility.

Gonadotropin-releasing hormone (GnRH) analog is frequently administered as a cotreatment in young cancer patients that possibly reduces gonadotoxicity. However, GnRH analog has been reported to cause loss of follicular pool and thus this approach is criticized. Chemotherapy can lead to damage of the primordial follicle pool and cause menopause and decreased fertility at a younger age. Although menstrual cyclicity and even ovulation have been found to be resumed, benefits in terms of reproductive outcome are controversial. The GnRH agonist causes suppression of the gonadotropin levels to prepubertal levels and decreases utero-ovarian perfusion; these actions are believed to protect the follicles from destruction.[48] Upregulation of antiapoptotic molecules, such as S1P has also been linked with the use of GnRH analogs.[49] Cochrane review of 2011 also concluded that GnRH analogs should be used in reproductive age group.[50]

Embryo Cryopreservation

The best time for fertility preservation method is considered to be before application of any chemotherapy. In young patients undergoing cancer treatment, the best option is cryopreservation of gametes. Married women can undergo ovulation induction followed by follicular aspiration and fertilization with the husband's sperm. Pregnancies can be achieved even years following the embryos' cryopreservation.[51] Pregnancy rate can be expected above 35% per transfer in these patients. It is proposed that preservation of 10 frozen embryos is sufficient to ensure future live birth.[52]

Embryo cryopreservation is an established technique with good pregnancy rates. However, collection of mature oocytes which involves ovarian stimulation is considered feasible only for adult women.[28,53] Furthermore, a stable relationship is also one of the requirement in these women. Also, in women with estrogen-sensitive tumors, ovarian stimulation is not advisable.

Oocyte Cryopreservation

Mature female oocytes are extremely fragile, because of their large size, water content, and chromosomal architecture. The spindle apparatus of the chromosome is easily damaged by intracellular ice formation during the freezing or thawing process.[54] In contrast, the immature oocytes are dormant, less differentiated, and without zona, and thus can tolerate cryopreservation better.[18,19]

Vitrification of oocytes is an important option for preserving fertility in patients who are unmarried or single.[55,56] However, this may lead to a delay of about 3 weeks in commencement of chemotherapy to enable follicular growth and follicular aspiration. Recently, in European Society of Human Reproduction and Embryology (ESHRE) 2008, vitrification of oocytes has been shown to be promising with more than 90% survival rate, though slow freezing of oocytes appears to be a golden standard in many centers with varying survival rates. Oocytes may be obtained after simulation with gonadotropins in cancers which are not sensitive to estrogen. Otherwise, immature oocytes could be collected and subjected to *in vitro* maturation (IVM) followed by cryopreservation. Alternatively, oocytes can be obtained and cryopreserved from biopsy of ovarian tissue or the whole ovary. If the patient had a partner, these oocytes could be fertilized after IVM and the resulting embryos could be frozen.[57,58] However, chances of pregnancy are limited by the number of oocytes or embryos stored. Oocyte cryopreservation, once deemed experimental due to the technical challenges associated with the size and structural complexity of oocytes, has now seen a higher success in several programs as evidenced by recent literature. With the use of cryoprotectants and cryotools in combination with rapid freezing techniques (vitrification) and fertilization with intracytoplasmic sperm injection (ICSI), multiple clinics have reported increased pregnancy rates using frozen and thawed oocytes.[59,60]

A few hundred children worldwide were born following cryopreservation of unfertilized oocytes. About 15 to 20 oocytes are considered as sufficient to be vitrified to ensure future child birth. Follow-up of the children born following this technique appears to be normal.[61]

The Practice Committee of the American Society for Reproductive Medicine, after reviewing available evidence, concluded that oocyte cryopreservation may be a viable alternative for those women with high potential for ovarian failure for whom embryo freezing is not an option.[62]

LIMITATIONS

Despite the obvious advantages, there are some issues in this approach. Firstly, ART is time-consuming and there is not much time between diagnosis and treatment of a cancer patient. Secondly, in prepubertal girls and patients without partners, ART is not advisable. Thirdly, ovarian stimulation using gonadotropins may not be suitable in women with

estrogen-dependent tumors, such as breast cancer. Oocyte freezing can be offered as an option in such conditions before commencing the treatment. Furthermore, numerous primordial follicles in the ovary can get wasted, if oocyte or embryo cryopreservation alone is done.

CONCLUSION

In the present scenario, OTC is still considered experimental, although more than 30 live births have been reported so far. It can be recommended in carefully selected patients and should be offered only by centers with the necessary laboratory and surgical expertise. The uncertainties, and the unknown effectiveness of the procedure for the restoration of fertility, dictate that it should only be offered to girls and young women who are at high risk of premature ovarian insufficiency and who will have a substantially reduced opportunity for fertility.

Accurate identification of these patients requires the assessment of the risk of premature ovarian insufficiency at the time of diagnosis itself, which in turn is affected by the nature of the treatment and not the disease. However, in children, the full consequences of treatment with respect to reproductive function are not fully known and will take several decades to be fully realized.[63,64] Thus an accurate fertility prognosis cannot always be predicted before treatment.

Factors that affect the assessment of an individual patient for invasive fertility preservation techniques can be grouped as intrinsic (i.e., related to the patient herself and her present state of health) and extrinsic (i.e., related largely to the anticipated treatment and the availability of appropriate expertise for the techniques proposed).[65] With the now proven success of OTC in adult women, it is important to establish that this experimental technique can be offered to the patients who are at high risk for the development of premature ovarian insufficiency, can safely undergo laparoscopic surgery, and have a good long-term prognosis.[34]

The major issues involving the OTC include optimal cryopreservation technique, length of time for which these can be kept in storage, optimal site of transplantation, expected survival of the thawed tissue, chance of regaining hormonal function, and chance of pregnancy after transplant.

Due to lack of appropriately designed peer-reviewed published studies performed by several independent investigators, including a description of materials and methods so as to assess scientific validity and to allow independent verification, the American Society for Reproductive Medicine does not recommend OTC as a standard procedure.

The account given so far clearly shows that OTC is still experimental, although pregnancies from this technique have been reported. Therefore, counseling occupies a very important role. Patients should be explained thoroughly that this technique is presently experimental and also that there is always a theoretical risk of reintroducing cancerous cells. All the available information and future prospects on OTC should be provided to the parents if the patient is a minor. After minor patients attain adulthood, they can decide upon using the tissue themselves. Getting informed consent after conveying the relevant medical information, explaining the risks and uncertainties, would help adult patients and parents of minor patients to avail the advantages of OTC.

Oocyte and embryo cryopreservation are now established techniques but have their limitations. However, OTC presents a chance of wider application along with the advantage of keeping the fertility window open for a longer duration. The need for fertility preservation has to be weighed against morbidity and mortality associated with cancer. Thus, there is a need for a multidisciplinary collaboration between oncologists and reproductive specialists to improve awareness and availability of different fertility restoring options according to the need and desire of patients.

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