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# Fertility Preservation in Female Cancer Patients: A Review

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**Abstract**

Cancer ART is one of the fast developing subspecialties in the ever expanding field of Assisted Reproductive Techniques. It is well documented that both chemotherapy and radiotherapy can have a deleterious effect on the gonads. Onco-ART targets this unfortunate population and provides a chance to the patient to produce their biological offspring. Cryopreservation of the ovaries is a step towards this goal. A properly preserved ovarian cortex can be later grafted in the body to enhance fertility. Even though this novel technique is still in its infancy stage, it holds a lot of promise that can be favorably explored.

**Keywords:** Ovarian cortex, cryopreservation, malignancy.

## INTRODUCTION

Newer modalities of radiotherapy and chemotherapy have remarkably increased the survival rates of young cancer survivor patients. Studies have shown that by 2010 one in 250 individuals will be a survivor of childhood cancer.[1] Unfortunately being gonadotoxic these treatments are associated with variable damage to the reproductive organs. Moreover the increase in the disease free life post-treatment raises concern about the outcome of such therapies on the fertility and feminity of the ever increasing young cancer survivors.[2] The risk for premature ovarian failure/dysfunction depends on the age of the patient, the type, and dose of chemotherapy, and the irradiation field used. The gonadotoxicity of these treatments can not only induce premature ovarian failure in patients but are also associated with various perinatal complications like abortions, premature deliveries and low birth weight at latter date.

The following review will evaluate the presently available reproductive options in young patients with emphasis on role of ovarian tissue freezing as emerging option for fertility preservation in young cancer patients.

## INDICATIONS OF OFFERING FERTILITY PRESERVATION

Fertility preservation is offered to young cancer patients planned for chemotherapy and or radiotherapy and those requiring hematopoietic stem cell transplant (HSCT) in varied malignant and nonmalignant diseases.[3]

Breast malignancy is the most common cancer of the reproductive age group. 15% of breast cancer occurs under the age of 45 years and majority of these cancers require multiagent cyclophosphmide based therapy which is gonadotoxic. Occult metastasis is rare (15%) in infiltrating lobular carcinoma. As compared to that of range of 70% in invasive ductal carcinoma thus making them poor candidates for ovarian tissue freezing. These patients can be subjected to the ovulation induction as the time interval between the surgery and the chemotherapy is 6 weeks. Occult metastasis is very rare in nonmetastatic breast cancers thus ovarian tissue cryopreservation may be recommended.[4]

Other common childhood tumors of the pelvis –Ewing sarcoma, osteosarcoma, retroperitoneal sarcomas, and anorectal cancers respond to radiation therapy which is gonadotoxic. Wilms tumor, neuroblastoma, rhabdomyosarcoma require cytotoxic chemotherapy thus compromising the reproductive potential. They can be offered oocyte, embryo, or ovarian tissue cryopreservation.

SLE and autoimmune disorders like Behcets disease, steroid resistant glomurelonephritis also benefit from cytotoxic therapy with alkylaing agents and thus are candidates for fertility preservation.

Stem cell transplant has been routinely being used presently for treatment of nonmalignant diseases, such as benign hematological disease (sickle cell anemia, thalassemia major and aplastic anemia) and autoimmune diseases (systemic lupus erythematosus and autoimmune thrombocytopenia) ,which do not respond to immunosuppressive therapy.

Prophylactic oopherectomy is offered to young girls with Turner syndrome and those with family history of premature ovarian failure. Inherited mutations –BRCA 1 and 2 account for nearly 10% of epithelial ovarian cancers. Life time risk for breast cancer for these patients' is 80-90%. These patients whose

family is incomplete or who want to postpone pregnancy after 35 years can be advised ovarian tissue freezing. They can be latter offered *in vitro* maturation of the follicles derived from the cryopreserved ovarian tissue. Lately young patients with benign ovarian tumors and pelvic endometriosis are being offered fertility preservation options.

Cervical cancer is a serious health problem and majority of cases are now occurring in third decade of life since past few years. It has been noted that the incidence of adenocarcinoma is increasing as compared to incidence of squamous cell carcinoma which has decreased by nearly 42%. Ovarian metastasis which is rare in squamous cell carcinoma is encountered in nearly 12% of the cases with adenocarcinoma of the cervix. Patients which require radiotherapy/chemotherapy will benefit from ovarian transposition and ovarian tissue freezing in nonmetastatic cervical cancers.

## VULNERABILITY OF THE REPRODUCTIVE SYSTEM TO CANCER TREATMENT

Radiotherapy and chemotherapy agents are toxic to cancer cells as well to the cells of the body. Successful treatment may be associated with long-term physiological dysfunction and reproductive sequel in the young cancer survivors.[5]

Tissue and the cells vulnerable to radio and chemotherapy are germ cells, sex steroid producing cells, cells in hypothalamus and pituitary glands. Damage to the tissues and vascular supply of the reproductive tract has also been documented.

Poiroit et al reported that before the age of 10 years there were 350-400 follicles/$mm^3$ but these reduced to 30-35/$mm^3$ between 25-34 years of age. This explains the depletion of follicles in elderly patients and more incidence of iatrogenic ovarian failure.[6,7]

## MODE OF ACTION OF GONADOTOXIC AGENTS

Radiotherapy causes irreversible cell damage by causing double stranded DNA breaks. Chemotherapy has got multiprong effect and hampers the normal cellular proliferation cycle by cross linking DNA strands, inhibiting purine, pyrimidine synthesis,dissociation of the microtubules,tyrosine kinase inhibition and causing inhibition of the transcription. Both the therapies can cause apoptosis induction in the gonadal tissues.

### Radiotherapy Induced Gonadal Damage

Females are exposed to irradiation in treatment of abdomino-pelvic malignancies, craniospinal irradiation for malignancies of central nervous system as acute lymphocytic leukemias and for total body irradiation as used for conditioning before stem cell transplant.

The nature of radiotherapy induced gonadal damage depends on the radiation field, total dose administered, and age of the patient. It has been noted that single dose is more gonadotoxic than fractioned dose. The human oocyte is sensitive to radiation, with an estimated LD50 of less than 2 Gy.[8] The irradiation threshold for POF is around 300 cGy as 63% of women developed POF above this threshold.[9] A single dose of irradiation between 7-8 Gy will cause POF in majority of postpubertal women. Chromosomal condensation, cytoplasmic vacoulations, and disruption of nuclear membrane are the common cellular changes observed on microscopy.

### Chemotherapy Induced Gonadal Damage

The impact of combination cytotoxic chemotherapy on gonadal function is dependent on age of the female undertaking treatment, the class and dosage of the drugs administered. Drugs known to cause definite gonadal toxicity include and the alkylating agents, particularly cyclophosphamide, chlorambucil, mustine, melphalan, busulphan, procarbazine, and nitrosoureas. Apoptosis in the pregranulosa cells along with disruption of the primordial follicle architecture in the ovarian cortical tissue in *in vitro* conditions has been documented histologically.[10]

Successful pregnancy outcome, with no increased risk of perinatal complications has been reported following treatment with multiagent chemotherapy regimens.

## ASSESSMENT OF OVARIAN RESERVE

Iatrogenic premature ovarian failure is a common consequence of gonadotoxic chemo and or radiotherapy. Inhibin B levels reduce postgonadotoxic treatment due to damage to granulosa cells. Reduced Inhibin B levels lead to increase in FSH levels as it has negative feed back effect on the pituitary gland. This is also associated with increase in serum estradiol levels initially which gradually fall to postmenopausal levels.

Antimullerian hormone (AMH) is another peptide produced by granulosa cells. A low level of AMH irrespective of the cycle day is marker of low ovarian reserve.[11]

Clomiphene challenge test and assessment of antral follicle count by the transvaginal ultrasonography are also carried out to asses the ovarian reserve.

## FERTILITY PRESERVATION OPTIONS

Fertility preservation in young cancer patients involves embryo cryopreservation, oocyte cryopreservation or ovarian cortex tissue cryopreservation. These options are presently available to preserve fertility in cancer patients and give them the opportunity to parent their children when there treatment is over. The selection of the Assisted Reproductive Technologies suitable for preserving fertility depends on the following parameters: the type and timing of chemotherapy/radiotherapy, the type, and site of malignancy, the patient's age, and the partner availability.

The purpose of these cryobiology techniques is to maintain viability of tissue after long-term storage at very low temperature. Cryopreservation requires gradual cooling of cells/ovarian tissue from 37°C to extremely low temperature of –196°C using programmable freezer, storage at this temperature, and then rewarming to 37°C when required. Lowering of the

temperature of the cell/tissue below the freezing point causes the water to expand as it changes its state to ice. This expansion can damage the integrity of the cell membranes, nuclear material, and essential organelles. This can be prevented by the use of permeating or nonpermeating cryoprotectants.

The conventional method for freezing embryos is called the slow freeze method, and is a technique that can also be used for freezing embryos, oocytes, and ovarian cortex tissue. Cryoprotectants such as dimethyl sulfoxide (DMSO) and sucrose are used. The temperature is lowered at a very slow rate of about –32°C per minute until reaching –32°C, at which point the sample is immersed in liquid nitrogen where it is rapidly cooled to –196°C. Seeding is carried out at –6°C to avoid supercooling of the solution.

Vitrification (rapid freeze) is an alternative strategy for cryopreservation and involves the rapid freezing of the sample at the rate of –30,000°C/minute. Higher concentrations of permeating cryoprotectants are used and contact period between the cells and cryoprotectant is very short to avoid toxicity. The sample is quickly immersed in liquid nitrogen and stored. Rapid thawing is required with this technique to prevent ice crystal formation.

### Embryo Preservation

Embryo preservation has excellent success rates and long-term data to support the safety of the procedure, the post-thaw survival rates of embryo is 35-90%, implantation rates are between 8-30% and cumulative pregnancy rates are over 60%,[12]

Embryo freezing is the only established program in human fertility preservation program. Unfortunately this has certain limitations which have to be kept in mind while advising this protocol—

a. When the initiation of the chemotherapy/radiotherapy cannot be delayed and there is no time to complete ovarian stimulation. Majority of the malignancies do not permit the oncologist to delay the treatment for carrying out IVF. Here use of antagonist protocol using GnRH antagonist is recommended which requires 10-12 days for follicular recruitment as compared to traditional long protocol with GnRH agonist which requires minimum of 3 weeks of hormonal therapy.
b. Conventional COH protocols are not recommended in patients with estrogen-sensitive breast cancers. Due to the formation of multiple mature follicles the circulating estradiol concentrations may exceed 3,000 pg/ml which is significantly greater than that of a natural, unstimulated cycle with peak estradiol levels of about 250-300 pg/ml. This hyper-estrogenemia can be a concern for these patients. It has been proposed to add tamoxifen or aromatase inhibitors (letrozole) to conventional gonadotrophin based IVF stimulation cycles.[13]
c. The availability of partner of the patient is essential for embryo cryopreservation program.
d. This technique is inappropriate for children, who have not attained puberty.

In conclusion, embryo cryopreservation is an efficient technique for patients from whom mature oocytes can be collected and who have a partner or are willing for donor sperm insemination.

### Oocyte Cryopreservation

Oocyte cryopreservation is yet another option for young patients with malignancy who are single and are/or not willing to inseminate their eggs using donated sperm. Drawback of this technology is that it is not applicable to applicable to children and young girls'. Human oocyte cryopreservation technology has been rapidly integrated into clinical practice. Seemingly very promising the outcome of this technique is not encouraging, with pregnancy and delivery rates ranging from 1 to 5% per frozen oocyte.[14]

Chilling injury to the oocyte modifies the structure of cell membranes and therefore disrupts their integrity**.** It also affects oocyte microtubular assembly, cytoskeletal organization, and cause hardening of the zona pellucida.[15]

Human oocytes can be retrieved and cryopreserved in mature or as immature state.

### Mature Oocyte Cryopreservation

The metaphase II (MII) oocyte is a large and highly specialized cell that is extremely fragile. Cryopreservation of the oocyte is associated with the zona pellucida hardening probably as a consequence of premature exocytosis of the cortical granules. This then act as a barrier, impairing sperm penetration and normal fertilization.

Secondly, in the mature oocyte, the metaphase chromosomes are lined up by the meiotic spindle along the equatorial plate, but the spindle apparatus is easily damaged by intracellular ice formation during the freezing/thawing procedure. The cooling associated with the technique causes depolymerization of the meiotic spindle, which is a dynamic structure with microtubules being continually assembled at one of its ends and separated at the other. Ultrarapid freezing with vitrification may offer advantages over conventional slow cooling protocols by improving post-thawing survival rates but needs to be investigated further.

### Immature Oocyte Cryopreservation

To overcome the paucity of time requirements necessary for harvesting mature oocytes in cancer patients and probably for avoiding chilling and freezing injuries during freezing mature oocytes, a newer strategy for immature oocyte freezing has been documented. This involves harvesting immature oocytes from unstimulated or minimally stimulated ovaries. Oocytes at the diplotene stage of prophase I, or germinal vesicle (GV) stage, survive the cryopreservation procedure better than those frozen at the MII stage**.** These immature oocytes have attained mature

size and complete meiotic competence but have not yet resumed their maturation process and initiated their second metaphase. Although the risk of hardening of the zona pellucida or damage to the cytoskeleton cannot be avoided, it is probable that the presence of a nuclear membrane protecting the chromatin protect the cell from cytogenetic anomalies.[16,17]

### Cryopreservation of Ovarian Tissue

Cryopreservation of ovarian tissue is an experimental option available for prepubertal girls, and for woman who cannot delay the start of chemotherapy/radiotherapy. Ovarian cortex can be cryopreserved as strips of cortex or as isolated primordial follicles. Whole ovary also may be cryopreserved with the vascular pedicle and transplanted at latter date. Human ovarian cryopreservation and transplantation procedures have so far been almost exclusively limited to avascular cortical strips, with the aim to reimplant cortical ovarian tissue into the pelvic cavity or a heterotopic site like the forearm or the abdominal wall once treatment is completed and the patient is disease-free.[18]

The ovarian cortex is cryopreserved because this part of the ovary is particularly rich in primordial follicles. The drawback of this technology is a decrease in primordial follicles within the grafted tissue due to hypoxia from a delay in revascularization of the graft and damage to the ovarian cortex while freezing. The loss of primordial follicles in cryopreserved ovarian cortex strips ranges from 50 to over 90%. The loss of ovarian function is corroborated with elevated FSH levels and low inhibin B levels after retransplantation.

Ovarian tissue harvesting and freezing can be carried out at any time during the menstrual cycle, without delaying chemotherapy or radiation therapy. After the transplantation of the graft in addition to fertility benefits, adequate estrogen may be produced though temporarily to treat menopausal symptoms and prevent the onset of osteoporosis.

### Ovarian Tissue Cryopreservation in Humans: Background

Ovarian tissue cryopreservation and transplantation studies date back to 1950's. results have improved over past few years due to ever increasing research in cryoprotectants and better cryofreezing equipment. The initial work in humans was done by Hovatta et al. He demonstrated that human ovary is cryo-resistant to freeze thaw protocol. He compared ovarian tissue freezing using DMSO and combination of 1-2 propendiol (PROH) and sucrose and found no difference in ovarian tissue morphology by histopathological examination with both the cryoprotectants.[19] Similar studies were carried out by Newton who noted that primordial follicles and ovarian cortex could sustain cryofreeze procedure.[20]

The first orthotopic transplantation was performed by Oktay and Karlikaya.[21] They reported harvesting and transplant of cortex in a 29-year-old patient that had undergone bilateral oophorectomy for a nonmalignant disease. Strips of tissue were thawed and sutured beneath the pelvic peritoneum by laparoscopy. They could establish blood flow and ovulation from the graft.

Heterotopic reimplantation of human ovarian tissue was performed by Leporrier et al.[22] They reported heterotopic transplantation of it ovary in a Hodgkin's patient. The ovary was transplanted to the arm subcutaneously before the radiotherapy initiation. Accidental subcutaneous transplantation of ovarian tissue was reported by Marconi et al[23] when, during a laparoscopic resection of an endometrioma, a piece of ovarian tissue was left in the subcutaneous area. Several weeks latter patient developed swelling along the umbilical region. Histopathological examination revealed functional ovarian tissue. Heterotopic transplantation of fresh tissue to the forearm was performed in two patients by Oktay et al.[24] He reports doing percutaneous oocyte aspiration with *in vitro* maturation and ICSI.

Successful pregnancies and deliveries have been documented in human in past 6 years. The first live birth in a patient with Hodgkin's lymphoma from frozen-thawed ovarian cortex after autologous orthotopic transplantation was reported by Donnez.[25] Ovarian cortex was cryopreserved before gonadotoxic treatment thawed cortical strips were transplanted into the peritoneal window beneath the hilum of the inactive right ovary. Pregnancy from *in vitro* fertilization in a modified natural cycle after the transplantation of cryopreserved ovarian tissue also resulted in live birth as reported by Meirow D.[26] Another spontaneous pregnancy was reported from orthotopic transplantation of frozen-thawed ovarian tissue by Demeestere I.[27] Spontaneous pregnancy with live birth by from transplantation of ovarian cortical graft in monozygotic twins has been reported by sibler.[28] fresh ovarian tissue was harvested from one healthy sister and transplanted to her monozygotic twin, who was having premature ovarian failure, which led to spontaneous pregnancy and latter live birth.

### Limitations for Ovarian Tissue Cryopreservation

A foremost impediment for the safe clinical application of ovarian tissue transplantation is the potential risk of transmission of cryopreserved cancerous cells that would reseed the malignancy in a cured patient. Cancer transmission depends upon the type of cancer. Systemic cancers are more likely to spread hematogenously and be present in the vascular channels in the ovarian tissue and reseed at the time of engraftment. Hodgkin's lymphoma, Wilm's tumor, lymphomas, osteosarcomas, Squamous cell cervical cancer; Ewing's sarcoma, and extragenital rhabdomyosarcomas rarely affect ovarian tissue and present low risk.

Adenocarcinoma and breast cancer have a moderate risk, but leukemia and neuroblastomas are aggressive and have a high-risk of spreading at the time of transplantation.

The development of technology to screen malignancy in the frozen tissue is important. Till date the only reliable technology has been histopathological examination of the ovarian cortex tissue for exclusion of Reed-Sternberg cells.

Sensitive molecular test like PCR studies which can detect a single tumor cell among $>10^5$ cells, immunohistochemistry and Northern or slot-blot analysis to detect myeloperoxidase expression in acute myeloblastic leukemia should be carried out at the time of ovarian cortex freezing to avoid reintroduction of cancerous cells.

## Methodology of Ovarian Tissue Collection and Processing of the Tissue

The ovarian resection is done laparoscopically and no special technique is required to remove the ovary. As electrocoagulation causes thermal damage to the ovary it should not be used. This helps in preserving more cortical tissue as well as gives chance to the patient to have spontaneous pregnancy from *in situ* ovary.

Ovaries of children are very small and delicate. These would be used nearly after 20 years in bioreposition so adequate precaution should be taken while excising and transporting them.

Immediately after extraction of the ovary/cortical tissue it should be immersed in transport media. HEPES based media without phenol red may be used. Ensure that the media has been kept at 4°C on ice. Gook and her team[29] kept the tissue at 37°C. Many commercial Medias are recommended for transport of the tissue. Leibovitz L-15 (Life technologies), HEPES-buffered Ham F-10 medium, ferticult HEPES buffered medium has been used by various workers.

When harvested ovary is received in the laboratory the time from the excision should be noted. An immediate assessment of the ovarian size should be done and number of strips to be prepared are planned. Accordingly ensure that our cryovials with the media are ready and are at 4°C. I draw your attention to excellent work by Dr Catherine P et al[9] about the recommended size of the cortical strips to be cryopreserved.

The ovary should be thoroughly washed in the IVF (bicarbonated) media and medulla/stromal tissue, removed doing sharp dissection. Ensure that the thickness of the cortex is even and it is clean. Cut adequate number of pieces and load 1 strip per cryovials. The thickness of the slices is kept optimal to facilitate equilibration of the cryoprotectant.

## Cortical Tissue Permeation with Cryoprotectants

Now these cryovials which contain strip of the ovarian cortex immersed in cryoprotectant solution comprising of 1. 5 M DMSO and 0. 1 M sucrose and 10% patient's serum. The vial is kept in ice and placed on shaker for gentle equilibration for 30 minutes[9,28] before they are moved to the programer.

Addition of a nonpermeating cryoprotectant (sucrose) may prevent cryoinjury to the ovarian tissue and act as osmotic buffer against the stress caused by the permeating cryoprotectant.

## Freezing and Thawing Protocols

Ovarian tissue freezing is done using programmable freezers available commercially. The point of seeding temperature is determined by the type of cryoprotectant solutions and may be carried out manually or automatically. The start point of the program depends on the type of cryoprotectant being used. Protocols using DMSO or ethylene glycol start freezing program at 4°C while those using PROH start near 20°C. This is done due to variable permeation coefficient of these cryoprotectants at these temperatures. Seeding temperature varies from –6°C to –9°C. The program is similar in most of the reports with a cooling rate of –2°C/minute to the seeding temperature followed by –0.3°C/minute to –40°C, –50°C/minute to –150°C and storage in liquid nitrogen.

Many different techniques have been recommended worldwide for thawing of the ovarian tissue. These all have common steps which involve keeping the vial at room temperature for 30 sec to 1 minute and wiping it clean with tissue. They are now immersed in 37°C water bath for 1-2 minutes. The vials can also be kept at the 37°C in the incubator if hot water bath is not available. Few studies have recommended rapid thawing of the vials by immersing them in the water bath for 2-3 minutes.

Now the cryoprotectants are gradually removed using lower concentrations of DMSO in 3-4 steps for 5 minutes each. These washing solutions contain 20% patient's serum without sucrose. After the removal of the cryoprotectants the ovarian cortex is transferred to the operation theater in HEPES based transport solution for transplant.

## Cryopreservation of the Whole Ovary

The main drawback of ovarian tissue (cortical strips) cryopreservation followed by avascular transplantation is that the engraftment is wholly dependent on the initiation of neovascularization which takes time. This results in a great proportion of primordial follicles being lost. This damage occurs primarily during the initial ischemia which occurs in early post-transplantation period.[30] Cryopreservation of the whole ovary has been proposed as an alternative to freezing ovarian cortical strips. Transplanting the intact ovary may avoid ischemic damage and maintain viability and function of the tissue though it is a challenge, due to its size and different cryoprotectant permeability coefficient of the heterogeneous tissues in ovarian cortical strip. Martinez-Madrid et al reports a cryopreservation protocol for intact human ovary with its vascular pedicle which has 75.1% survival rates of follicles. No induction of apoptosis was observed in any cell types, assessed by both the terminal deoxynucleotidyl transferase biotin-dUTP nick-end labelling (TUNEL) method and immunohistochemistry for active caspase-3.[31] Transmission electron microscopy revealed 96.7% intact primordial follicles post-thaw.

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.

### Role of Vitrification in Human Ovarian Tissue Cryopreservation

Till date majority of the work done on ovarian cortex studies has been by slow freezing technologies. All the pregnancies reported from human ovarian cryopreservation have resulted from slow freezing of ovarian tissue. Studies have documented that rapid freezing of ovarian tissue resulted in a lower proportion of intact oocytes and a higher proportion of vacuolated oocytes. Attempts to vitrify human ovarian tissue have not given acceptable results due to the increase in necrosis observed in vitrified human ovarian tissue.

## ETHICAL CONCERNS IN OVARIAN TISSUE CRYOPRESERVATION

Ovarian tissue cryopreservation is a promising mean of fertility preservation. In the present scenario the procedure is still experimental, although pregnancies from this technique have been reported. It is the moral responsibility of the team coordinator that the procedure does not harm the patient by delaying cancer treatment and all attempts should be made to ensure that no remnant malignancy cells are reintroduced by subsequent transplantation. The selection of cases should be carried out by multidisciplinary team including oncologists, gynecologists, reproductive biologists, and psychologists. Parents should be given all available information and future prospects on ovarian tissue cryopreservation if the patient is a minor.

## NOVEL OPTIONS OF FERTILITY PRESERVATION

### Ovarian Transposition

Ovarian transposition can be used as a strategy that avoids danger to the ovary without removing it from the body. The ovarian dose after transposition is reduced to approximately 5-10% of that in the untransposed ovaries.[32] Lateral ovarian transposition is more effective than transposing the ovaries behind the uterus and protecting them with a lead block which may also shield affected nodes.

Lateral ovarian transposition is typically performed by laparotomy at the time of radical hysterectomy for cervical cancer or staging laparotomy for Hodgkin's disease.

In a study it was confirmed that ovarian function was preserved if they were transposed at least 3 cm from the upper border of the field or above the iliac crest. Even after transposition, ovarian failure may result if the ovaries are not moved far enough out of the radiation field or if they migrate back to their original position.[33]

### Medical Options

The medical approach to female fertility preservation entails temporary induction of a prepubertal hormonal milieu, such as the use of GnRH analogues or the use of antiapoptotic agents to hinder loss of ovarian follicles.

A number of studies have shown that GnRH analogues inhibit chemotherapy induced ovarian follicular depletion in rodents by blocking gonadotrophin induction. These findings are supported by clinical studies by Blumenfeld Z which showed that cotreatment of GnRH analogues and chemotherapy in lymphoma patients resulted in primary ovarian failure in 1 of 44 (2.3%) compared with 26 of 45 (58%) in the group treated with chemotherapy (with or without mantle field irradiation) only.[34] In contrast, a randomized controlled study, using intranasal buserelin before chemotherapy, failed to preserve fertility in patients with Hodgkin's disease.[35]. At present, there is no consensus about the efficacy of GnRH analogues in preventing chemotherapy induced POF.

Oocyte loss induced by cytotoxic therapy has been shown to occur by mechanism of apoptosis. Sphingosine-1-phosphate (S1P), a metabolite of ceramide, is believed to inhibit apoptosis in somatic cells further studies however are necessary to explore the detrimental effects of this treatment on normal neurological function.

## CONCLUSION

With the management of childhood and pubertal age malignancies becoming gradually more successful the undesirable effects of treatment on reproductive function are assuming greater importance. Preservation of fertility before treatment must be considered in all young patients at high-risk of fertility compromise, and option must be offered to parents and child by oncology centers and assisted reproductive biology units. The rapidly advancing experimental techniques for harvesting of gonadal tissue must be considered without unrealistic expectations as future utilization of the tissue is unlikely to be realized until the next decade.

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