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## Present and Future Fertility Preservation Strategies for Female Cancer Patients

Elena S. Georgescu, MD,\* Jeffrey M. Goldberg, MD,†  
Stefan S. du Plessis, PhD, MBA,‡ and Ashok Agarwal, PhD, HCLD§

\*Research Fellow, Center for Reproductive Medicine, Cleveland Clinic, Ohio; †Head, Section of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Cleveland Clinic, Ohio; ‡Senior Lecturer, Division of Medical Physiology, University of Stellenbosch, Tygerberg, South Africa; §Professor and Director, Center for Reproductive Medicine, Glickman Urological and Kidney Institute and Ob-Gyn and Women's Health Institute, Cleveland Clinic, Ohio

As survival rates with cancer treatment are steadily increasing, many women are now facing sterility due to treatment induced ovarian failure. This review will attempt to summarize the options for trying to preserve fertility in these patients. The optimal approach depends on the type of cancer, the type of treatment (e.g., radiation and/or chemotherapy), time available till onset of treatment, patient's age, and whether the patient has a partner.

Ovarian transposition remains the standard of care for women undergoing pelvic radiation, although it has been suggested that it may be combined with ovarian tissue cryopreservation. For patients about to receive chemotherapy or whole body radiation, *in vitro* fertilization (IVF) with embryo cryopreservation is a well established treatment with a good success rate. However, it requires delaying cancer treatment for 2 to 4 weeks and a partner or willingness to use donor sperm. When these criteria cannot be met, more experimental options include oocyte cryopreservation for later IVF and ovarian tissue cryopreservation. The tissue may be autotransplanted back to the pelvis, when the patient is in remission, to attempt spontaneous conception or subcutaneously for easy access of follicle aspiration for IVF. Alternatively, it may be xenografted to immunocompromised mice to induce follicle maturation in preparation for retrieval for IVF.

Emerging treatment options for fertility preservation include medication to prevent chemotherapy-induced oocyte damage and oocyte construction from somatic cell nuclei. IVF with donor oocyte remains an established option with a very high success rate for those who fail to conceive with the above measures or who elect not to avail themselves to experimental procedures.

**Target Audience:** Obstetricians & Gynecologists, Family Physicians

**Learning Objectives:** After completion of this article, the reader should be able to demonstrate knowledge about fertility preservation when counseling appropriate female cancer patients, recall current clinical strategies to assist women cancer patients to try to maintain their fertility if they wish, and appraise future strategies as they develop.

Fertility preservation in female patients has become a very topical issue. It has been estimated that in the United States alone, more than 650,000 women were afflicted with cancer in 2003 (1) of which, 8% were <40 years old. Moreover, cancer is estimated to occur in 113 per 100,000 women under the age of 50 each year in the United States. Breast cancer is the

most common malignant disease in women of reproductive age (2). It is estimated that 15% of all breast cancer cases will occur in women 40 years and younger (3). Childhood cancers are the second highest leading cause of death in children between the ages of 1 and 14 years worldwide (3). By 2010, it is estimated that 1 in every 250 people in the adult

population will be childhood cancer survivors (4). Many patients are therefore at risk of ovarian failure as a consequence of radiation therapy or treatment with cytotoxic chemotherapy.

Cancer treatment-induced ovarian function not only puts the patients at risk for menopause-related complications at a very young age but is also associated with fertility loss. For those females not sterilized by radiation or chemotherapy, there may be an increased risk of complications during pregnancy such as early pregnancy loss, premature labor, and low birth weight (5). With the emergence of several options to preserve fertility, together with an increased awareness of their availability, a greater number of patients are being offered and utilizing them (6). The options for preserving fertility range from clinically established to experimental to futuristic techniques. It is the aim of this review to provide the reader with an overview of these methods.

### CHEMOTHERAPY- AND RADIOTHERAPY- ASSOCIATED DAMAGE

Multiagent chemotherapy constitutes the basis of treatment for many cancers. The ovaries have an irreplaceable number of follicles and are extremely sensitive to cytotoxic drugs that induce irreversible gonadal damage. Structurally, this is normally associated with marked follicle loss (7). According to Tauchmanova et al. (8) some of the chemotherapeutic agents most associated with gonadal damage are cyclophosphamide, chlorambucil, melphalan, busulfan, nitrogen mustard, and procarbazine. Cyclophosphamide is the agent most often implicated in damaging the oocytes and granulosa cells in a dose-dependent manner (9). Cisplatin and adriamycin are considered to be moderately gonadotoxic (10). Bleomycin, actinomycin D, vincristin, methotrexate and 5-fluorouracil are associated with mild or no gonadotoxicity (11).

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Reprint requests to: Dr. Ashok Agarwal, Reproductive Research Center, Glickman Urological and Kidney Institute and Department of Obstetrics-Gynecology, Cleveland Clinic, 9500 Euclid Ave., Desk A19.1, Cleveland, OH 44195. E-mail: agarwaa@ccf.org.

Ionizing radiation is a well-recognized cause of ovarian damage and permanent infertility. The risk is significant for patients exposed to pelvic radiation. Up to 32% experience decreased fertility. Radiation with a dose of more than 6 Gy directly over the ovary usually leads to permanent infertility. Although successful full term pregnancies were reported (12), the risk of ovarian failure is high in women who receive high-dose abdominal and pelvic radiation (13). Moreover, as stated primarily, if pregnancy is achieved, these patients have increased risks for complications, including early pregnancy loss, premature labor, and low birth weight due to impaired uterine growth and blood flow (5).

### OPTIONS FOR FERTILITY PRESERVATION

Cancer treatment may result in diminished ovarian reserve or complete ovarian failure (14). At present, there is a limited number of established and reliable treatments; however various pharmacological-, surgical-, and laboratory-based treatments are emerging with plausible outcomes (Table 1).

#### Pharmacologic Treatment

##### *Ovarian Suppression*

Rendering ovarian function quiescent as a treatment modality for fertility preservation, before and during anticancer treatments, still remains a topic of debate. Blumenfeld and Hain (15) first showed that gonadal damage could be minimized or prevented during cytotoxic therapy by suppressing ovarian function via a gonadotropin-releasing hormone (GnRH) agonist which inhibits pituitary gonadotropin secretion. Treatment should begin 10 days before the start of chemotherapy and continue throughout the treatment period (16,17). It has been proposed that GnRH antagonists instead of agonists could be used in future for the faster achievement of pituitary-ovarian desensitization (17).

TABLE 1  
Different strategies for fertility preservation

Established	Emerging	Revolutionary
Gonadal shielding	Ovarian suppression	Apoptotic inhibitors
Ovarian transposition	Oocyte cryopreservation	Xenografting cryopreserved ovarian tissue
Embryo cryobanking	Ovarian tissue cryopreservation	Reconstructed oocytes
Donor oocytes		

Various other studies failed to achieve any protective effect from GnRH treatment (18,19). The lack of conclusive evidence to support the safety and efficacy of GnRH analogue treatment was recently reviewed by Oktay et al (20). Oehninger (14) advised that because the benefits, long-term effects and putative mechanism of action of GnRH analogue administration are not clearly elucidated, it should only be given as part of prospective research and under ethics committee-approved protocols.

In a retrospective study in younger women undergoing gonadotoxic chemotherapy, a possible protective effect of oral contraceptives was found (21). The oral contraceptive pill suppresses pituitary gonadotropin secretion, thereby inhibiting follicular growth (16). Confirmatory prospective studies are needed before this can be recommended to patients, but its low cost, lack of significant side-effects, and risks makes it appealing for patients with no contraindication to receiving ovarian hormones.

### Apoptotic Inhibitors

Apoptosis is a normal phenomenon in both male and female germ cells. Genetic programming and numerous cellular pathways are involved in this process (22). Various chemotherapeutic drugs can activate these apoptotic pathways, leading to oocyte depletion and premature ovarian failure (POF). A known apoptosis inhibitor, sphingosine-1-phosphate (s1p), restricts developmental death of oocytes during mouse fetal ovarian gametogenesis. S1p furthermore was able to protect oocytes from undergoing apoptosis when exposed to a known apoptosis trigger (doxorubicin) and preserve fertility of irradiated female mice *in vitro* (23). Further studies showed that s1p treatment of female mice before radiation therapy did not cause genomic damage in their offspring (24).

Taking all of the former data into account, the use of apoptosis inhibitors could potentially be used as a pharmacological tool to stop radiation- and chemotherapy-induced apoptosis and thus protects the patient from POF. As many chemotherapeutic agents work via apoptosis at the tumor level, further studies are needed to establish the effectiveness of apoptosis inhibitors for fertility preservation and assurance that cure rates will not be compromised.

### OVARIAN TRANSPOSITION

Cervical, vaginal and anorectal carcinomas, dysgerminoma, Hodgkin's disease, and some CNS tumors

may affect premenopausal women and are curable with radiation therapy. However, the standard radiation dose to the ovaries with pelvic radiation therapy uniformly induces ovarian failure. Surgically transposing the ovaries above the pelvic brim, out of the radiation field, before pelvic radiation therapy reduces radiation exposure to the ovaries to only 5 to 10% of nontransposed ovaries (25). For women under age 40, 88.6% retained ovarian function and 89% of pregnancies were spontaneous with 75% occurring without repositioning the ovaries. There was no increase in the rates of stillbirth, low birth weight, congenital malformations, abnormal karyotypes, or cancer in the offspring (26,27). The ovaries would need to be mobilized back to the pelvis before IVF.

Ovarian transposition is usually performed laparoscopically by dividing the utero-ovarian ligaments and the mesovarium to separate the ovary from the uterus and tubes. The ovarian vessels are mobilized to transpose the ovaries above the pelvic brim without tension. The ovaries are brought through a peritoneal tunnel such that the ovaries remain intraperitoneal, but the vessels are kept retroperitoneal to reduce the risk of kinking or torsion, which could compromise the ovarian blood supply. Permanent suture is used to secure the ovaries to the peritoneum and surgical clips are applied to the ovaries so that they can be located on x-ray before radiation treatment. Recently, it was suggested that only 1 ovary should be transposed while the other is removed for cryopreservation, thereby minimizing the risk of single treatment failure (28).

### Cryopreservation

Cryopreservation, the maintenance of the viability of excised tissue or organs by storing them at very low temperatures, opened various possibilities for preserving fertility by combining it with surgical techniques and/or IVF.

#### *Embryo Cryopreservation*

To date, ovarian transposition, IVF with donor oocytes, and IVF with embryo cryopreservation represent the only established methods for preservation of child-bearing potential in women at risk of gonadal failure. Cancer patients can undergo IVF treatment, and the resulting embryos can be cryopreserved and stored for future use. The survival rates per thawed embryo range from 35% to 90% and implantation rates from 8% to 30%. Thus, the cumulative pregnancy rates can be >60% (29). The overall pregnancy rate with transfer of cryo-

preserved embryos in the United States in 2005 according to the Society for Assisted Reproductive Technology (SART) was 28% versus 34% with fresh embryos (30).

Time constraint is the biggest limitation of embryo cryopreservation as there may not be enough time for controlled ovarian hyperstimulation and oocyte retrieval, usually 2 to 3 weeks before the onset of chemotherapy. The second factor to be considered is whether the patient has a partner, willing to enter into IVF treatment, and embryo cryopreservation. Alternatively, the woman may elect to use donor sperm. The third factor is the concern about supraphysiologic estradiol levels from controlled ovarian hyperstimulation in patients with estrogen-dependent tumors such as breast cancer. The use of letrozole, an aromatase inhibitor, in combination with gonadotropins has been proposed as a means to reduce the estradiol levels in these patients (20). Finally, the patients should sign an advanced directive indicating whether the embryos should be donated to another couple, used for research, or discarded in the event that she does not survive her cancer or otherwise elects not to have them transferred.

#### *Oocyte Cryopreservation*

Cryopreservation of unfertilized oocytes is an option for women without a partner who opt not to use donor sperm for IVF, but it remains an investigational procedure. Oocytes are extremely sensitive to cryoinjury, and the meiotic spindle, cytoskeleton, cortical granules, and zona pellucida are at particular risk (31). A recent meta-analysis reported that Although the success rates from the programs with the most experience have greatly improved in the past few years, the live-birth rates with conventional slow-freezing protocols were significantly lower than IVF with fresh oocytes, 15.4% versus 38.4%, respectively (32). Cryopreservation utilizing vitrification, an ultra-rapid freezing protocol, which avoids ice crystal formation in the cytoplasm, approaches the live birth rates with fresh oocytes (32).

Pregnancies after oocyte cryopreservation occurred with mature oocytes, which require 2 to 3 weeks for induction of multiple follicular maturation and places the patient at risk of ovarian hyperstimulation syndrome. *In vitro* fertilization with *in vitro* maturation (IVM) of immature oocytes obtained during a spontaneous menstrual cycle avoids those problems and has been shown to yield comparable pregnancy rates to conventional IVF cycles (33). IVM is still best suited for patients with polycystic ovaries containing numerous small antral follicles. There have only been 2 live

births with IVF using IVM and oocyte cryopreservation. The first was achieved with IVM after cryopreservation of immature oocytes (34). The second resulted from IVM with vitrification of the matured oocytes (Tan SL et al, unpublished data). It has also been suggested that IVM and oocyte vitrification may be performed with oocytes aspirated from antral follicles of ovarian tissue removed for cryopreservation (35).

#### *Ovarian Tissue Cryopreservation*

When embryo or oocyte cryopreservation is not feasible, another experimental technique, ovarian tissue cryopreservation, may be an option. Ovarian tissue can be harvested at laparoscopy or laparotomy by performing several ovarian biopsies, or partial uni- or bi-lateral oophorectomy. Small pieces of ovarian cortical tissue are cryopreserved for later transfer after the patient is in remission.

The advantages of ovarian tissue cryopreservation include the following: the tissue can be obtained without delay because there is no need for ovarian stimulation, no partner is required for male gamete donation at the time of tissue harvesting, and finally, the transplanted tissue will resume the production of endogenous hormones obviating the need for hormone replacement therapy. Ovarian tissue cryopreservation is not an option for women with ovarian cancer. It is less clear whether it should be utilized in women who have an estrogen-dependent cancer. The disadvantages are that it requires surgical procedures for tissue harvesting and transfer and there have been only a few pregnancies world-wide.

The ovarian cortex has an advantage over antral follicle oocytes for being cryopreserved. The oocytes within the primordial follicles are arrested at the diplotene stage of prophase of the first meiotic division and have a relatively high surface/volume ratio, low metabolic rate, and the absence of zona pellucida, which make them less susceptible to cryodamage. Vitrification may also improve success with ovarian tissue cryopreservation but is still in its initial stages. A study comparing fresh, vitrified, and slow-freeze cryopreservation found more morphologically intact primordial follicles in the vitrification group than the slow-freeze group, 80.3% versus 72.6%, respectively. Both groups secreted estradiol and progesterone continuously during 14 days of *in vitro* culture (36).

The resumption of ovarian endocrine function after cryopreservation and transplantation has been demonstrated in women (37,38). Recently, the first human embryo produced by IVF after subcutaneous

heterotopic transplantation of cryopreserved ovarian tissue (39) and 2 human pregnancies after orthotopic transplantation were reported (40). These isolated successes must be considered very preliminary and the technique itself experimental. It also remains to be determined what the optimal method should be for transplantation (41). The options for transplanting the thawed ovarian tissue are discussed below.

### *Xenograft Transplant*

The tissue may be xenografted to severe combined immunodeficient (SCID) mice intramuscularly, subcutaneously, or under the kidney capsule to improve vascularization. Although immature oocytes can mature and ovulate in these xenotransplanted animals, aberrant microtubule organization and chromatin patterns observed during the maturation process are of significant concern (42,43). The advantages of this method are the avoidance of further surgery to transplant the tissue back to the patient and eliminating the potential risk of reintroducing cancer cells. There have been no clinical pregnancies with xenografting to date.

### *Autotransplantation*

There are 2 approaches to autotransplanting the ovarian cortical pieces back to the patient, orthotopic, and heterotopic transplantation. During the orthotopic transplant procedure, strips of ovarian tissue can be transplanted to the ovarian fossa or on a nonfunctional ovary. Alternatively, in heterotopic transplantation, the ovarian tissue is grafted subcutaneously at various locations including the forearm and abdominal wall. Heterotopic transplantation would be preferred to better monitor the ovaries when there is concern for growth of metastatic cells or malignant transformation. There have been 2 healthy children born after orthotopic ovarian tissue autotransplantation (40,44). These reports have been surrounded with controversy about the source of the fertilized oocytes as the retained ovaries may still have been functional (41). Rosendahl et al (45) reported that a biochemical pregnancy was achieved with IVF using oocytes obtained from heterotopic autotransplantation of cryopreserved ovarian tissue.

The functional life span of the transplanted tissue seems to be about 3 years. This is because only a small amount of tissue is transferred and follicle attrition occurs from ischemic injury until the tissue develops a collateral blood supply. This has led to interest in the cryopreservation of whole ovaries

with vascular anastomosis. In a very recent study by Martinez-Madrid et al, no evidence of apoptosis or ultrastructural alterations was noted following a slow-freeze cryopreservation and rapid thaw protocol of whole human ovaries with their vascular pedicle, obtained from 3 women (46).

In women with nonovarian-related cancer, there is still a theoretical risk of reimplanting metastatic cells originating from the primary tumor during the autotransplantation procedure. Some cancers such as blood borne malignancies, leukemia, Burkitt lymphoma, and neuroblastoma may metastasize to ovaries. Transmission of lymphoma via an ovarian tissue graft from diseased donor mice to healthy recipients has been reported (47). However, the majority of tumors encountered in reproductive age have a low potential to metastasize to the ovaries (48). Another risk of ovarian tissue transplantation is the malignant transformation of the transplanted ovary in women with the BRCA1 and BRCA mutations. Mutations in the suppressor genes BRCA1 and BRCA2 predispose women to ovarian and breast cancer, and an orthotopic transplantation approach should not be offered to these patients.

### **Donor Oocytes and Artificial Gametes**

A considerable number of women are unable to avail themselves of the above options due to lack of a male partner or sufficient time to undergo IVF. The risk of reintroducing cancer cells with autologous ovarian tissue transfer may be unacceptable. Many patients are also unwilling to utilize experimental treatments, especially those requiring surgery. Finally, older women have a much poorer prognosis with any of these treatments. For these patients, IVF with donor oocytes provides a standard treatment with very high success rates. The pregnancy rate in the United States in 2005 according to SART was 52.3% (30).

The use of donor oocytes may lead to legal, ethical, and emotional problems (49). These issues, plus the high cost of treatment, often discourage cancer survivors from choosing this option. Donors may also be reluctant to undergo weeks of daily injections, the inconvenience of frequent follicle monitoring, and the risk of ovarian hyperstimulation syndrome associated with controlled ovarian hyperstimulation to induce multiple follicular maturation. The use of IVM with natural cycles will make the donor oocyte option more affordable and may increase the willingness of women to donate oocytes by eliminating the risks and inconvenience. This treatment was recently

shown to have comparable success rates to conventional donor oocyte IVF cycles (50).

Emerging reproductive technologies might lead to alternative sources of gametes and embryos through which these infertile patients can finally conceive their own genetic child (49,51). Somatic cell nuclear transfer (cloning) has already been successfully performed in mammals, whereas the construction of reconstituted human oocytes and fertilization have been successfully attempted by Takeuchi et al. (52). A detailed review of the techniques has been published by Nagy and Chang (53). The process can be summarized briefly as removing the karyoplast of an immature oocyte at the germinal vesical stage and inserting a somatic cell into the perivitelline space of the enucleated oocyte. Finally electrofusion leads to union of the cells (54). By introducing this diploid cell into a cytoplasm that is preprogrammed to undergo meiosis, haploidization is achieved and subsequently an artificial oocyte (53). As the patients would not be able to provide their own oocytes, donor-enucleated oocytes must be used. This translates to the genetic information of the offspring originating from three sources; i.e., paternal, maternal, and donor ooplasm (mitochondrial DNA). This idea of creating artificially constructed oocytes is attainable but still in its early experimental phases; however, it might just provide the best solution for female cancer survivors to conceive their own genetic offspring.

### Other Indications for Fertility Preservation

Systemic lupus erythematosus and other autoimmune diseases such as Behcet disease, steroid-resistant glomerulonephritis, inflammatory bowel disease, and pemphigus vulgaris typically involve women of reproductive age (55). High dosages of alkylating agents like cyclophosphamide with or without hematopoietic stem cell transplantation are used to treat these conditions and also result in infertility due to ovarian failure (56). Hematopoietic stem cell transplantation is used to treat leukemias, lymphomas, and several nonmalignant conditions encountered during reproductive age including sickle cell anemia, myelodysplastic syndrome, multiple sclerosis, systemic sclerosis, rheumatoid arthritis, and juvenile chronic arthritis. Cyclophosphamide, busulfan/cyclophosphamide, or whole body irradiation is used to ablate the existing bone marrow treatment before hematopoietic stem cell transplantation (57). Each of these will almost certainly result in ovarian failure.

### CONCLUSIONS

Fortunately, modern chemotherapy and radiation therapy regimens have enabled many girls and reproductive age women to survive their cancers but at the cost of rendering them sterile due to ovarian failure. Currently, only ovarian transposition, conventional IVF with embryo cryopreservation, and IVF with donor oocytes are considered standard treatment op-

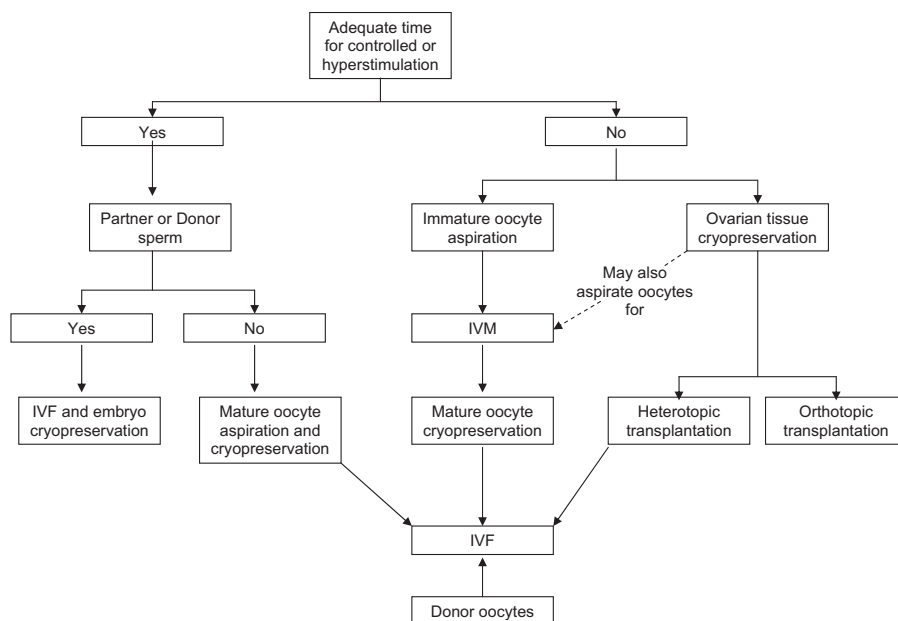


Fig. 1. Flow diagram outlining the decision analysis for fertility preservation options.

tions for fertility preservation with reasonable success rates. Medical treatment with gonadotropin analogs has yielded conflicting results, and apoptosis inhibitors have shown promise in preliminary animal studies.

Live birth rates continue to improve with IVF after oocyte cryopreservation primarily due to vitrification techniques. The combination of IVM with oocyte cryopreservation prevents delaying cancer treatment and avoids the cost, inconvenience, and risks associated with controlled ovarian hyperstimulation including exposure to suprphysiologic estradiol levels, which may further stimulate estrogen-dependent tumors. Ovarian tissue cryopreservation suffers from the need for surgery to harvest and transplant the tissue, very low success rates, and the risk of reintroducing malignant cells. Xenografting may eliminate some of these problems, but it is as yet unproven and will not likely be a popular choice for the patients. Whole ovary cryopreservation with vascular anastomosis would have the advantage of restoring not only fertility but long-term endogenous ovarian hormone production, but the difficulties of cryopreserving a large organ need to be overcome first.

There are many variables to take into consideration when deciding upon fertility preservation treatments (Fig. 1) such as the patient's age, cancer type and stage, proposed treatment regime and time before it is initiated, and availability of partner sperm (or willingness to use donor sperm). Issues for the patients include potential risks including surgical complications, ovarian hyperstimulation syndrome, delaying cancer treatment and reintroduction of cancer cells, cost, low success rates and experimental nature of the fertility preservation treatments, and the disposition of gametes and/or embryos in the event that the patient does not survive her cancer. It is very important for the physician to advise the patient of all of these factors so she may make an informed decision regarding her fertility preservation options. It is anticipated that when these fertility preservation treatments reach their full potential, all women will benefit not just those with cancer.

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