

## Preservation of Ovarian Function in the Cancer Patient

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Survival rates for cancers that occur in women of reproductive age have improved dramatically. Preservation of fertility in these women has become a more relevant issue. Many treatments that are administered for childhood and adolescent cancers carry a substantial risk for future infertility. This risk varies according to the presenting pathology and requires preventive treatment.

Treatment of Hodgkin's disease, breast cancer, rheumatoid arthritis and systemic lupus erythematosus need cytotoxic drugs and appear to be irreversible in the ovary. Depending on the type of chemotherapy regimen used the risk of gonadal damage increases with the age of the female. Prepubertal girls seem less susceptible than young women to cytotoxic drugs.

Alkylating agents such as cyclophosphamide, L-phenylalanine mustard, and chlorambucil permanently damage gonadal tissue by interacting chemically with DNA and protein synthesis is inhibited. Agents that do not induce permanent ovarian failure include 5-fluorouracil, etoposide, and doxorubicin. gonadal dysfunction has not been demonstrated in women who were treated with methotrexate.

Temporary amenorrhea will result when maturing follicles are destroyed by cytotoxic drugs. Permanent amenorrhea or Premature Ovarian Failure (POF) will result when all primordial follicles are destroyed. Xate.

### Pharmacologic Protection

- **Gonadotropin-Releasing Hormone Agonists (GnRH-a):**-Blumenfeld and colleagues<sup>1, 2, 3</sup> have reported on the largest group of females exposed to both cytotoxic drugs and GnRH-a. Sixty patients with lymphoma were started on GnRH-a 7-10 days before their chemotherapy regimen. The rate of POF was 5% in the GnRH-a/chemotherapy group versus 55% in the chemotherapy-alone group.

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- **Progesterone (P4):**-Familiari<sup>4</sup> evaluated the ultrastructural changes in the primordial follicles of humans exposed to cytotoxic drugs and medroxyprogesterone acetate (MPA). The number of follicles noted in the chemotherapy plus MPA group was smaller than that of the control plus MPA group (19.37±3.41/mm vs. 28.55±6.59/mm).

Using electron microscopy, they found an increased frequency of cellular features that are typically seen during early atresia in both the oocytes and the accompanying follicle cells<sup>4</sup>. These changes were seen 4-5 months after chemotherapy was completed. They concluded that chemotherapy not only acutely damaged the ovary by reducing the number of follicles, but also chronically damaged the quality of the follicles. These ultra-structural changes seem to indicate that factors produced by gonadal cells are modified by chemotherapeutic drugs, leading to a subsequent increase in rate of atresia. MPA was unable to protect the ovary from early follicular atresia.

- **Apoptotic Inhibitors:**-Because a series of specific signaling events are activated in the cell that is bound for apoptosis, inhibiting these signaling events could potentially stop the apoptotic process and protect the patient from POF.

Sphingosine-1-phosphate may be an example of an apoptotic inhibitor, and may some day play a role in preventing oocyte loss.

- **Oral Contraceptive Pills:**-Studies were unable to demonstrate protective effect on the ovaries.

### Surgical Transposition

The ovarian follicles are remarkably vulnerable to DNA damage from ionizing radiation. Irradiation results in ovarian atrophy and reduced follicle stores<sup>5</sup>. On the cellular level, oocytes show rapid onset of pyknosis, chromosome

condensation, disruption of the nuclear envelope, and cytoplasmic vacuolization. Serum levels of FSH and LH rise progressively and serum estradiol (E<sub>2</sub>) levels decline within 4-8 weeks after radiation exposure<sup>6</sup>. The degree and persistence of ovarian damage and suppression of ovarian function is related to the patient's age and the dose of radiation delivered to the ovaries<sup>7, 8</sup> (Table 1). Two studies indicated that the cutoff for radiation-induced ovarian failure is around 300 cGy. Only 11%-13% experienced ovarian failure below 300 cGy versus 60%-63% above that threshold value (44). Adding chemotherapy increases the risk of POP<sup>9, 10</sup>.

Ovarian Dose (cGy)	Results
60	No deleterious effect
150	No deleterious effect in young women; some risk for sterilization in women older than 40
250-500	In women aged 15-40, 60% permanently sterilized; remainder may suffer temporary amenorrhea. In women older than 40, 100% permanently sterilized
500-800	In women aged 15-40, 60%-70% permanently sterilized; remainder may experience temporary amenorrhea. No data available for women over 40
>800	100% permanently sterilized

**Note: From Damewood and Grochow (Damewood and Grochow, 1996) Falcone. Ovarian function preservation. Fertil Steril 2004.**

To improve quality of life and preserve fertility in these young women, ovarian function has been maintained for over three decades by transposing the ovaries out of the field of irradiation. The ovarian dose after transposition is reduced to approximately 5%-10% of that in the untransposed ovaries<sup>11, 12, 13</sup>.

Lateral ovarian transposition is typically performed by laparotomy at the time of radical hysterectomy for cervical cancer or staging laparotomy for Hodgkin's disease. The utero-ovarian ligament is divided, and the ovary is mobilized with the ovarian vessels to the paracolic gutters. Ideally, the vascular pedicles are kept retroperitoneal to avoid tension, torsion, or trauma and bowel herniation while the ovaries remain intraperitoneal to reduce cyst formation<sup>14</sup>.

Bidzinski<sup>15</sup> 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. 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. Ovarian failure after transposition may also be due to compromised ovarian vessels from the surgical technique or radiation injury to the vascular pedicle<sup>16</sup>.

Another concern with ovarian transposition is the development of symptomatic ovarian cysts. The mechanism that causes the cysts is unknown<sup>17</sup>. The transposed ovaries can be followed with computed tomography and ultrasound<sup>18, 19</sup>. Symptomatic ovarian cysts requiring reoperation develop in 1%-5.2% of patients after hysterectomy for benign disease and in 4.9%-7.6% of patients after radical hysterectomy only<sup>16, 17</sup>. For the above reasons, ovarian transposition should be performed laparoscopically just before the start of radiation therapy. Radiation therapy can be initiated immediately after surgery, thereby avoiding ovarian migration and failure<sup>10, 20, 21</sup>. When vaginal or cervical cancers are treated with brachytherapy, laparoscopic ovarian transposition can be performed under the same anesthesia<sup>8</sup> interestingly, 89% of the pregnancies were spontaneous, with 75% occurring without ovary repositioning. The ovaries were only repositioned in cases of infertility, and 11% of those patients conceived with IVF<sup>22</sup>.

Tulandi<sup>23</sup> reported a case of laparoscopic lateral ovarian transposition in a patient with rectal adenocarcinoma whereby the utero-ovarian ligaments were divided but the ovaries remained attached to the distal fallopian tubes, which potentially improved the chances for ovum pickup. The patient achieved a spontaneous pregnancy<sup>24</sup>.

Covens<sup>13</sup> and Swerdlow<sup>25</sup> confirmed that there were no excess cases of stillbirths, low birth weight, congenital malformations, abnormal karyotypes, or cancer in the offspring of women treated for Hodgkin's disease. However,<sup>26</sup> cited an increase in low birth weight and spontaneous abortions, especially if conception occurred less than a year after radiation exposure. They advised delaying pregnancy for a year after completing radiation therapy. To facilitate oocyte retrieval as well as the diagnosis and treatment of ovarian cysts. The ovaries have been transposed subcutaneously. This has the disadvantages of requiring a laparotomy and an additional abdominal incision and may also be associated with a higher rate of cyst formation. Transient

ovulatory pain was reported by 81.5% of the patients, and 15.4% required needle aspiration<sup>27</sup>

## **Cryopreservation of Oocytes, Embryos, and Ovarian Tissue**

### ***Oocyte Cryopreservation***

cryoprotectant. When comparing the results of using DMSO and PrOH as cryoprotectants, better survival was reported with PrOH<sup>28</sup>. Using the same technique<sup>29</sup>, reported 16 pregnancies that resulted in 11 live births from Cryopreservation of 1,796 oocytes. These results showed that despite early disappointing results regarding survival and fertilization and cleavage rates, the recent introduction of a technical modification may improve the clinical efficacy of this technology. It has been suggested by<sup>29</sup> and others that the routine use of intracytoplasmic sperm injection (ICSI) to achieve fertilization with cryopreserved oocytes has contributed significantly to the increased success reported in the studies. cryopreservation insult the lowest at the primordial follicle levels<sup>30</sup>. On the other hand, antral follicles contain oocytes either in the prophase I or M-II stage, and their oocytes are much more susceptible to cryodamage<sup>31</sup>.

### ***Mechanisms of Cryodamage***

- Meiotic spindle damage and chromosomal aberrations.
- Cytoskeleton: microtubules and microfilaments.
- Reduced number and morphological alterations of the cortical granules.
- Zona hardening resulting in failed fertilization and hatching.
- Organelle damage.
- Osmotic damage of the plasma membrane.

(Falcone. Ovarian function preservation. Fertil Steril 2004).

## **Cryopreservation of the Pre-implantation Human Embryo for Ovarian Function Protection**

Embryo cryopreservation was introduced to maximize the conception chances from a single cycle. The Society of Assisted Reproductive Technology has reported delivery rates per embryo transfer (ET) using cryopreserved embryos<sup>32</sup> to be 18.6%. This option may not be acceptable to prepubertal girls, adolescents, and

women without a partner. However, acceptable, long-term data are available about the outcome of children born from these procedures. Typically, a standard IVF protocol is used. Because of time

Recently, the use of tamoxifen - a nonsteroidal anti estrogen - has been investigated for ovarian stimulation for IVF<sup>33</sup>. Tamoxifen (40-60 mg) was started on day 2 or 3 of the cycle and given daily for 5-12 days. If a GnRH-antagonist was required, a low-dose gonadotropin was also given until follicular maturity. Embryos were frozen at the pronuclear stage. The tamoxifen group had a mean of 1.6 embryos versus 0.6 embryos for the natural cycle group. The peak E<sub>2</sub> concentration in the tamoxifen group was higher than the natural cycle group. Some researchers are investigating the use of aromatase inhibitors for use in these patients. These inhibitors are associated with much lower E<sub>2</sub> levels that may be critical for estrogen sensitive tumors.

## **Ovarian Tissue Cryopreservation and Transplantation**

Ovarian cryopreservation and transplantation is an experimental procedure that was introduced to preserve fertility in women with threatened reproductive potential<sup>33</sup>. Studies investigating the effects of cryopreservation insult on ovarian tissues have been limited compared with those studying the same effects on oocytes<sup>34</sup>. Unlike a suspended single cell, tissue cryopreservation presents serious physical constraints related to heat and mass transfer. Furthermore, because it is a multicellular structure for which cell to cell interactions are known to exist, the dynamics of cryoprotectant permeation into and out of the tissue during cryopreservation are of utmost importance for subsequent tissue survival.

## **Factors Affecting the Outcome of Ovarian Tissue Cryopreservation and Transplantation**

### ***Cryoprotectants (CPAs)***

Newton et al. demonstrated that human ovarian tissue lose 90% of the primordial follicle population when using glycerol as a CPA compared with 25%, 15%, and 55% using DMSO, ethylene glycol (EG), and propylene glycol (PG), respectively.

### ***Effect of Ischemia***

Ischemia is a critical factor in follicular loss and can potentially occur at different steps: during the stages of preparation of tissue for cryopreservation, during the stages of preparation for transplantation, and during the revascularization process after transplantation. According to size showed a significantly higher follicle count for larger tissue sections 5mm.

### ***Vascularized Versus Nonvascularized Graft***

Although whole ovaries from mice and rats survive freezing because of their smaller sizes, successful cryopreservation of whole ovaries from other mammalian species such as human and nonhuman primates is more technically challenging because of heat and mass transfer limitations as well as post-transplantation limitations.

Points of concern are blood flow, apoptotic signals, follicular viability, serum E<sub>2</sub>, FSH, and histologic evaluation. Research is now required to demonstrate its application in humans.

### **Potential Utility of Cryopreserved Ovarian Tissue: Xeno-Grafting of Human Ovarian Tissue**

#### ***Autografting of Human Ovarian Tissue***

It is critical for patients to understand that no pregnancies have been reported in humans with these techniques.

### **Conclusion**

Research should focus on refining cryopreservation protocols, cryoprotectants, and transplantation techniques that decrease ischemia. When selecting a transplantation site, physicians should consider whether it can be accessed using simple and minimally invasive surgery. Moreover, ample blood supply to the recipient site is important for graft establishment, survival, and long-term function. Subsequent manipulations of the grafted ovary as in follicle aspiration should be made simple. For all these reasons collectively, we are providing a hypothetical model for the potential recipient sites.

There are many options that are available to a patient undergoing a treatment that will negatively impact her fertility. Focused on the potential success and limitations of these techniques. Many procedures and medical interventions have proven success rates both in terms of ovarian function and pregnancy rates.

Other techniques have great potential but do not have long-term clinical data. It is important that the patient's primary care physician understand the available methods to preserve fertility in cancer patients and communicate this information to the patient.

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