

Ovarian tissue and oocyte cryopreservation

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Although currently investigational, ovarian tissue cryopreservation and oocyte cryopreservation hold promise for future female fertility preservation, particularly following aggressive chemotherapy and/or radiotherapy treatment protocols. (Fertil Steril® 2004;82:993–8. ©2004 by American Society for Reproductive Medicine.)

There are relatively few effective clinical options for preserving female fertility, particularly following aggressive chemotherapy and/or radiotherapy treatment protocols. This document reviews the scientific background, current technology, clinical results and potential future applications of two methods for preserving female fertility—ovarian tissue cryopreservation and oocyte cryopreservation. These technologies are investigational, although rapidly evolving, and their list of appropriate indications may be expanded in the future.

FEMALE FERTILITY PRESERVATION

Background

In 2001, over 625,000 women in the United States were diagnosed with some form of invasive cancer. Approximately 8%, or 50,000, of these women were under the age of 40 (1, 2). With current treatment regimens, including aggressive chemotherapy, radiotherapy, and bone marrow transplantation (BMT), cure rates for some malignancies can exceed 90% (3). However, the alkylating agents (e.g., busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, thiotepa) and ionizing radiation can often induce premature ovarian failure, rendering the patient infertile. Most female cancer patients of reproductive age do not have the option of utilizing established assisted reproductive technologies to safeguard their fertility. In nearly all cancers, with the possible exception of breast cancer, chemotherapy is initiated soon after diagnosis. Because preparation and stimulation for oocyte

retrieval usually requires 2 to 3 weeks or longer, it is generally not feasible to freeze embryos from an adult female cancer patient for potential future use. Even considering the frequent hiatus between surgery and chemotherapy in breast cancer patients, most would not be candidates for oocyte or embryo freezing due to concerns that high estrogen levels might have detrimental effects on the primary tumor. Additionally, not all patients have partners with whom they can create embryos to cryopreserve.

Most female cancer patients therefore have limited clinical options for fertility preservation. In select cases, an oophorectomy may be performed to move an ovary out of an intended radiation therapy field. Treatment with gonadotropin-releasing hormone (GnRH) analogs or oral contraceptives during chemotherapy has been advocated to protect the female gonad, although convincing evidence of benefit is yet to be seen. Embryo banking is a proven method but requires both available sperm and several weeks of preparation. Oocyte banking avoids some of the disadvantages of embryo banking, although investigations of the application of this technology have been hampered historically by poor oocyte survival, fertilization, and resulting pregnancy rates. Recently, there have been more encouraging reports on the outcomes of oocyte freezing. Ovarian tissue banking has been successful in restoring fertility in laboratory animals and in at least one human. Ovarian tissue banking remains a promising clinical technique because it avoids ovarian stimulation and provides the opportunity for preserving gonadal function in prepubertal, as well as adult patients.

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Potential Indications

Ovarian cryopreservation and auto-transplantation were initially designed to protect and restore reproductive function in female cancer patients receiving sterilizing chemotherapy and/or radiotherapy. The current possibilities for ovarian cryopreservation extend beyond cancer, as gonadotoxic chemotherapy is being used in a number of benign systemic diseases as well. Moreover, patients undergoing oophorectomy for benign ovarian conditions or for prophylaxis potentially may benefit from ovarian cryopreservation. The primary experimental indications for ovarian tissue cryopreservation or oocyte cryopreservation (if time permits) for fertility preservation, both in cancer and non-cancer patients, are as follows:

Patients Receiving Chemo- and/or Radiotherapy for Treatment of Cancer

As noted, more than 50,000 reproductive-age women will be diagnosed with cancer in the United States each year. Breast cancer is the most common cancer seen in reproductive-age women. Approximately 15% of the estimated 182,000 annual cases of invasive breast cancer in the United States will occur in women less than 45 years of age (3). Many of these patients opt for adjunctive chemotherapy and, as a result, are at risk for premature ovarian failure.

Approximately 3,000 cases of cervical cancer will also occur in premenopausal women annually (4). Patients who require adjunctive therapies for cervical cancer may similarly face a risk of premature ovarian failure. Survival rates have increased dramatically for children with typical cancers of childhood and youth (5) with treatment regimens that include chemotherapy, radiotherapy and BMT. Overall, the five-year survival rate has increased from less than 30% to nearly 70% (2). As a result of these more effective treatment regimens, more than 4,000 female children are exposed to potentially sterilizing chemotherapy and/or radiotherapy in the United States annually.

Patients Undergoing Bone Marrow (BMT) or Stem Cell Transplantation (SCT)

Whereas BMT was initially used as a treatment for patients with leukemia, it is now being increasingly utilized for a number of other cancerous and non-cancerous diseases. Prior to BMT or SCT, high doses of chemotherapy and/or radiotherapy are used to ablate the bone marrow. Unfortunately, this regimen results in ovarian failure in nearly all patients (6).

Oophorectomy for Benign Ovarian Tumors, Endometriosis, or Prophylaxis

Bilateral oophorectomy results in sterility.

Autoimmune Diseases

For patients with severe lupus carditis, a regimen of cyclophosphamide, a potentially sterilizing form of chemo-

therapy, is often initiated. Similarly, in other autoimmune diseases such as glomerulonephritis and Behçet's disease, cyclophosphamide treatment is used with increasing frequency, rendering patients infertile.

OVARIAN TISSUE CRYOPRESERVATION

Clinical Studies

Encouraged by the mounting evidence from animal ovarian autograft studies (7–9), similar techniques have been applied in humans. In theory, natural pregnancy might be achieved via orthotopic transplantation (an autograft placed near the infundibulopelvic ligament) if the fallopian tubes remain intact and the transplant does not become sequestered beneath the peritoneum. Although fresh ovarian transplantation was reported as early as 1906 (10), orthotopic transplantation of previously frozen ovarian tissue had been performed only in animals until 1999.

Trials in sheep had demonstrated that orthotopic transplantation techniques could restore ovarian endocrine function, fertility, and yield viable offspring (11). In humans, ovarian stimulation has successfully induced ovulation from transplanted frozen-thawed ovarian cortical tissue (12, 13). In one individual, transient restoration of spontaneous ovarian follicular development and estrogen production, but not ovulation, was observed after autotransplantation of frozen-thawed ovarian tissue that had been harvested and banked before chemotherapy and radiation therapy for lymphoma (14). The first human livebirth after orthotopic transplantation of cryopreserved ovarian tissue has now been reported, also in a woman previously treated with chemotherapy and radiation for lymphoma (15). Because sporadic spontaneous ovulation had been observed after treatment and before transplantation, it is also possible, but unlikely, that the pregnancy may have derived from an ovum released not from the transplant but from the ovaries which remained in situ.

There have been several additional reports regarding the use of heterotopic sites for ovarian transplantation. Utilizing a forearm heterotopic autograft, in which ovarian tissue is grafted into the subcutaneous space above the brachioradialis fascia of the forearm, patients must undergo an IVF-ET procedure to conceive. The forearm transplantation technique, however, does not require general anesthesia or abdominal surgery and allows the ovarian tissue to be closely monitored. If needed, ovarian removal would be less complicated with a forearm heterotopic graft rather than an orthotopic graft. Moreover, the forearm has been successfully used for autografting fresh and frozen-banked parathyroid tissue for many years (16, 17). Ovarian function has been restored in two patients for at least 2 years after transplanting ovarian tissue to the forearm. In one of these patients, oocytes were even aspirated percutaneously (18).

A recent primate study has also confirmed the feasibility of the forearm heterotopic approach in restoring menstrual

cyclicality and the capacity to produce mature oocytes in response to gonadotropin stimulation (19). The first live birth in a primate after heterotopic ovarian transplantation has been reported (20). In humans, a morphologically normal embryo that was transferred has been reported, but no pregnancy has resulted (21).

Because of the relatively low follicle survival rate currently seen after ovarian transplantation, it does not appear to be feasible to cryopreserve ovarian tissue from women older than 40 years of age (22). In patients younger than 40, the amount of ovarian tissue cryopreserved theoretically 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.

Potential Risks

There is a legitimate concern regarding the potential for reseeding tumor cells following ovarian transplantation procedures in cancer patients. Although many types of cancer virtually never metastasize to the ovaries, leukemias are systemic in nature and pose a significant risk. Neuroblastomas and breast cancers are also of moderate risk to metastasize to the ovaries. On the other hand, ovarian involvement is extremely rare in Wilms' tumor, lymphomas (with the exception of Burkitt's lymphoma), osteosarcomas, Ewing's sarcoma, and extragenital rhabdomyosarcomas. Ovarian involvement is also highly unlikely in squamous cell cervical cancers, even in the most advanced stages.

In order to minimize the risk of cryopreserving ovarian tissue with metastases, histological evaluation always should be performed on multiple harvested ovarian tissue samples. In cases of leukemia or lymphoma, chromosomal and other tumor markers can be studied by immunohistochemical or other molecular biological methods to screen for the presence of cancer cells (23). Prior to undertaking ovarian tissue cryopreservation, a consultation with the patient's medical oncologist is always appropriate.

The potential for malignant transformation of the transplanted ovarian tissue raises an interesting dilemma. In cases where oophorectomy is performed in patients with an increased risk of ovarian cancer due to a genetic predisposition (i.e., mutation in tumor suppressor genes or BRCA-1 or BRCA-2 mutation), replacing the same tissue could pose significant risks for future malignancy. If one assumes that the risk of ovarian cancer will be suspended at the age of ovary removal and the oophorectomy is done at a young age, the tissue could perhaps be transplanted to a heterotopic site and removed as soon as pregnancy is achieved, potentially exposing the patient to a shorter interval of risk. The safety of such an approach for BRCA-positive patients needs to be determined.

There are no human studies that have specifically examined the quality of oocytes and embryos that result following a prior course of chemotherapy. It is known that chemotherapeutic agents can cause mutations, DNA adducts, and structural breaks, as well as oxidative damage in somatic and germ cells. Fertilization in female mice recently exposed to cyclophosphamide resulted in a higher rate of pregnancy failures and fetal malformations (24).

Studies that have examined pregnancy outcomes in cancer survivors have found no significant increase in congenital malformations or malignant neoplasms in the resulting offspring (25, 26). These studies, however, primarily evaluated women who conceived years after their chemotherapy treatment. (Until human studies are available, and depending on the age of the patient and type of cancer being treated, it is suggested that patients avoid attempting pregnancy for at least 3 to 6 months after chemotherapy. What is a safe interval after completing chemotherapy prior to ovarian tissue harvesting and cryopreservation is unknown at present.)

Conclusion

Ovarian tissue cryopreservation and transplantation is experimental. Future research in larger numbers of patients will determine whether acceptable longevity can be achieved with both pelvic and forearm ovarian cortical transplant procedures and whether fertility reliably can be restored. Research should focus on better defining patient suitability, methods of tissue collection, optimal tissue size, choice of cryoprotectants and cryopreservation protocols, and possible *in vitro* maturation of oocytes for human ovarian tissue. In addition, research is needed in order to enhance the revascularization process with the goal of reducing the follicular loss that takes place after tissue grafting.

Currently, ovarian tissue cryopreservation can only be recommended as an experimental protocol in carefully selected patients. This is important to emphasize as there appears to be a growing trend for centers to offer ovarian tissue cryopreservation solely for the potential of future use. Because many unanswered questions remain regarding who is an appropriate candidate for the procedure as well as the optimal methods of tissue collection and cryopreservation, ovarian tissue freezing itself, even without transplantation, should still be considered experimental. Likewise, ovarian transplantation procedures should only be performed as experimental procedures in centers under institutional review board (IRB) guidelines. These procedures (either ovarian tissue cryopreservation or transplantation) should not be advertised as established clinical services offered by assisted reproduction programs. Appropriate current experimental indications primarily focus on providing an alternative for women who immediately face near term medical therapies that clearly threaten their future fertility.

Due to the present potential risk-to-benefit ratio, ovarian tissue cryopreservation should not be currently either marketed or offered as a means to defer reproductive aging.

OOCYTE CRYOPRESERVATION

Background

Oocyte cryopreservation is another experimental option for female fertility preservation. Oocyte cryopreservation is an attractive strategy as it does not require surgery, and well-tested stimulation protocols for IVF can be used. Unfortunately, the majority of cancer patients do not have enough time to complete an IVF stimulation cycle before starting cancer treatment. In addition, pregnancy rates after transfer of thawed fertilized oocytes have been quite low. Recently, however, several studies have reported better post-thaw oocyte survival, fertilization, and pregnancy rates (27, 28). For this reason, a renewed interest in oocyte cryopreservation has occurred.

The metaphase-II oocyte is extremely fragile due to its large size, water content, and chromosomal arrangement. In the mature oocyte, the metaphase chromosomes are lined up by the meiotic spindle along the equatorial plate. It has been well documented that the spindle apparatus is easily damaged by intracellular ice formation during the freezing or thawing process (29, 30). In addition, hardening of the zona pellucida can adversely affect the normal fertilization process (31).

Laboratory Methods

In oocytes, rates of freezing and thawing damage differ according to maturational stage (32, 33). Oocytes frozen at the germinal vesicle (GV) stage survive better than those frozen at the metaphase-II stage (34). Additionally, oocytes frozen at the GV stage have lower rates of abnormalities in the resulting meiotic spindle than oocytes frozen at the metaphase-II stage (30). Even though GV oocytes have a superior thaw survival rate and a lower incidence of meiotic spindle damage, the continued inefficiency of *in vitro* maturation protocols results in a final yield of mature oocytes that is similar to that obtained with cryopreserved metaphase-II oocytes. Variability in survival rates of frozen-thawed metaphase-II oocytes may be partly attributable to the quality of oocytes used (35).

Other studies have suggested that modifications in the cryopreservation methods are responsible for these differences. Increasing the sucrose concentration of the cryoprotectant medium, for example, increased the survival rate of frozen M-II oocytes in a dose-dependent manner (36). Numerous studies have also reported improved oocyte survival by modifications of cryopreservation techniques such as changing the initial temperature of the cryoprotectant (37), changing the seeding temperature (38), using low-sodium medium (39), or injecting cryoprotectants (e.g., trehalose) directly into the oocyte (40). The use of intracytoplasmic sperm injection (ICSI) has additionally improved fertilization rates and overcomes the issue of potential zona hardening after freezing (41, 42).

Vitrification is the process of cryopreservation using high concentrations of cryoprotectant to solidify the cell into a glass-like state without the formation of ice. In humans,

post-thaw survival rates of vitrified oocytes have improved and fertilization rates are beginning to rival those of fresh oocytes (43). Compared to control oocytes, similar rates of maturation, fertilization, and embryo development have been obtained by vitrification of immature human oocytes (44). Human pregnancies and deliveries from vitrified mature oocytes have also been reported (43, 45).

Clinical Studies

To date, there remain a limited number of established pregnancies and deliveries derived from cryopreserved oocytes. No increase in the number of abnormal or stray chromosomes in thawed, previously cryopreserved oocytes has been observed (46). The incidence of chromosomal abnormalities in human embryos obtained from cryopreserved oocytes was no different from that of control embryos using fluorescence *in-situ* hybridization (47). One recent follow-up of 13 children resulting from cryopreserved oocytes failed to reveal any abnormalities in karyotype, mean age at delivery, mean birth weight, or organ formation (48). In another 3-year follow-up study of 16 children born after oocyte cryopreservation, one case of ventricular septal defect was noted (49). In the latter report, the investigators also failed to detect any intellectual or developmental deficits in any of the children.

Potential Risks

Due to the known effects of cryopreservation on the meiotic spindle of the oocyte, there remain concerns regarding the potential for chromosomal aneuploidy or other karyotypic abnormalities in the offspring. Concerns similarly remain regarding the potential for organ malformations or other developmental problems. Despite the few promising studies on vitrification, even less is known about the potentially detrimental effects of vitrification when compared with conventional cryopreservation techniques.

Conclusion

The experimental nature of oocyte cryopreservation suggests potential for clinical application, although it is too soon to conclude that the incidence of anomalies and developmental abnormalities of children born from cryopreserved oocytes are similar to those born from cryopreserved embryos. Oocyte cryopreservation will need to be studied in adequate numbers of patients for a sufficient length of time to determine whether the development of children is comparable to those conceived from other established assisted reproduction techniques.

Moreover, while the pregnancy rates might be improving, the current pregnancy rates appear to be significantly less than those seen with standard IVF procedures. In the case of patients who are facing infertility due to chemotherapy, oocyte cryopreservation may be one of the few options available. It might therefore be acceptable under these circumstances with appropriate informed consent in an investigational protocol under the auspices of an IRB. On the other hand, there is not yet sufficient data to recommend

oocyte cryopreservation for the sole purpose of circumventing reproductive aging in healthy women.

SUMMARY AND CONCLUSIONS

- Chemotherapy and radiotherapy pose significant risks to future female fertility.
- For women facing upcoming cancer therapies, there is generally insufficient time available to permit ovarian stimulation, oocyte retrieval, and embryo freezing.
- Ovarian tissue cryopreservation and oocyte cryopreservation hold promise for future female fertility preservation.
- Ovarian function has been documented in a small number of cases following both orthotopic (pelvic) and heterotopic (forearm, abdomen) transplantation of thawed ovarian cortical strips.
- Ovarian tissue cryopreservation or transplantation procedures should be performed only as experimental procedures under IRB guidelines.
- Recent laboratory modifications have resulted in improved oocyte survival, oocyte fertilization, and pregnancy rates from frozen-thawed oocytes in IVF.
- Although based on a limited number of established pregnancies and deliveries resulting from cryopreserved oocytes, no increase in chromosomal abnormalities, birth defects, or developmental deficits have been noted in the children born from cryopreserved oocytes to date.
- Oocyte cryopreservation presently should be considered an experimental technique only to be performed under investigational protocol under the auspices of an IRB.
- At the present time, neither ovarian tissue nor oocyte cryopreservation should be marketed or offered as a means to defer reproductive aging.

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