Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience

Kutluk Oktay, M.D., F.A.C.O.G., and Ozgur Oktay, M.D.


Objective: To assess the indications, safety, utilization, and success of ovarian tissue freezing and transplantation.

Design: Prospective longitudinal analysis.

Setting: Academic medical centers.

Patient(s): Fifty-nine women who underwent ovarian tissue cryopreservation with a slow freezing technique between May 1997 and March 2008. A follow-up was conducted 36.8 ± 3.6 months after the procedure.

Intervention(s): Ovarian tissue harvesting and cryopreservation.

Main Outcome Measure(s): Indications, safety, and utilization rates.

Result(s): The mean age (± SE) was 26.7 ± 1.2 years (range 4–44 years). The majority of patients had either hematologic malignancies (45.7%) or breast cancer (22%). Of these, 57.6% underwent hematopoietic stem cell transplantation. No complications occurred and no histologic evidence of cancer was found in the harvested tissue. The median length of storage was 3.5 ± 0.3 years (0.06–10.5 years). Fifty-six of 59 patients have not yet used their ovarian tissue. The reasons for nonutilization were social/personal, being still under treatment, and death in 54%, 38%, and 8%, respectively. Only three women (5.1%) underwent transplantation, two with the heterotopic (abdominal wall) and one with the orthotopic technique. One woman with a heterotopic transplant conceived spontaneously and delivered. Of the three transplants, one ceased function after 9 months and two are still functioning at up to 7 years follow-up.

Conclusion(s): Ovarian tissue harvesting appears to be safe but the experience with ovarian transplantation is still limited due to low utilization. As a result, the true value of this procedure remains to be determined. (Fertil Steril® 2010;93:762–8. ©2010 by American Society for Reproductive Medicine.)

Key Words: Fertility preservation, female, ovary, cryopreservation, cancer, chemotherapy, transplantation

Approximately 2% of women less than age 40 years will be diagnosed with invasive cancer and this incidence increases to 9% for women less than age 60 years (1). Nearly one-half of those women are estimated to receive a treatment that would impact reproductive function. This fact, coupled with the significant increase in survival rates (1), makes preservation of reproductive function one of the most pressing issues of young survivors. The American Society of Clinical Oncology has issued recent clinical guidelines encouraging counseling about fertility preservation among all young cancer survivors with interest in fertility (2). Likewise, the President’s Cancer Panel issued a strong recommendation to increase research on fertility preservation (3).

As a result of this increasing emphasis on fertility issues of young survivors, a number of fertility preservation techniques have been developed, and established techniques, such as embryo cryopreservation, are more frequently considered.

Embryo cryopreservation is the most established fertility preservation technique, as it has been used for nearly two decades to store unused embryos from IVF and embryo transfer cycles as options for fertility preservation (2). However, this technique requires at least 2 weeks from the beginning of the menstrual cycle, which may not be available to all patients with cancer. Furthermore, embryo cryopreservation requires a partner and ovarian stimulation, both of which are not possible in prepubertal girls. The same technical limitations also apply for oocyte cryopreservation where the only difference from embryo freezing is that a partner is not needed. However, success rates with oocyte cryopreservation are considerably lower compared with embryo freezing (4).

A more recently developed fertility preservation technique is ovarian cryopreservation. Ovarian cryopreservation research spans more than 50 years, but its application to human ovarian tissue is confined to the past decade (5). The first case of autologous ovarian transplantation with cryopreserved tissue was reported in 2000 (6). Likewise, first reports of embryo generation (7) and spontaneous pregnancies (8) after SC transplantation of frozen banked tissue date back only to 2004 and 2006. More recently, two live births after autologous ovarian transplantation to the pelvis have been reported (9, 10), although the origin of these pregnancies could not be confirmed with 100% certainty, as especially in the report by Donnez et al. (9), the patient continued to ovulate from the contralateral ovary.
Although highly experimental and not yet fully validated in clinical trials, ovarian cryopreservation is the only option for prepubertal children and those who do not have time to undergo ovarian stimulation for oocyte or embryo cryopreservation. Thus, we developed a clinical ovarian cryopreservation and transplantation program within the past decade under an Institutional Review Board (IRB)-approved protocol. However, offering an elective and experimental procedure to patients with cancer requires certain safeguards. At a minimum, the surgical procedure to remove the ovarian tissue should be sufficiently safe to be offered to study participants. Furthermore, as this procedure requires removal of ovarian tissue, the indications should be sufficiently strong to justify even the minimal risk associated with this surgical procedure. Therefore we prepared this report to answer the foregoing questions, and to describe our experience with ovarian cryopreservation and transplantation within the past decade. We hypothesized that ovarian cryopreservation is a safe procedure and is justified in patients with cancer and with a sufficiently high risk of developing ovarian failure.

**MATERIALS AND METHODS**

The study was approved by the IRBs at all institutions involved. Written consent was obtained from all patients or in the case of minors, from their guardians. Minors also provided assent. Data were obtained with the secondary review of the medical records of study subjects who underwent ovarian cryopreservation and transplantation; the final follow-up information was obtained prospectively.

**Harvesting of Ovarian Tissue**

Ovarian tissue harvesting was performed by laparoscopy in 52 of 59 cases. In the remaining seven cases, a separate indication existed for laparotomy, and in five of those, the primary surgeon performed ovarian tissue harvesting. All laparoscopic harvesting procedures were performed by a single surgeon (K.O.), except in four women, and in two of those, the tissue was frozen elsewhere and transported to our center. The laparoscopic procedure was performed by a three-puncture technique where, after general anesthesia, the abdomen was insufflated with CO₂ gas through a Verres needle inserted infraumbilically. Subsequently a 5-mm disposable port was inserted through the umbilicus and a 5-mm video laparoscope was introduced through this port. Under laparoscopic vision, two additional ports were inserted in the lower quadrants, 5 and 12 mm in size. The side for the larger port insertion was determined based on accessibility, and whether there was a large follicle or corpus luteum (CL) were preferred. The 12-mm trocar was inserted to the opposite quadrant of the ovary to be removed (see later for details of the surgical technique).

First, using a device that can both coagulate in a bipolar fashion and cut (Ligasure, Valleylab Inc., Boulder, CO), the utero-ovarian ligament was dissected. Then successive bites were taken to transect the mesosalpinx in parallel to the fallopian tube up to the point of infundibulopelvic ligament. At all times the tube was gently manipulated by a bowel grasper. By this approach the tube was preserved in case natural conception would be desired after future transplantation to this site. Once reaching the infundibulopelvic ligament, the ovarian vessels were skeletonized using fine graspers and microscissors and leaving a pedicle no thicker than 1 cm. Then the ovarian vessels were ligated with double 0-Vicryl endoloop applications; the highest suture placement was at least 1 cm away from the base of the ovary.

The ovary was then immediately placed in a laparoscopic specimen bag (Endo Catch, Autosuture, United States Surgical, Norwalk, CT) introduced through the 12-mm site, and the entire port was pulled out to remove the specimen from the abdomen. In some cases where the ovary did not fit through the 12-mm surgical incision, the incision was enlarged to allow smooth release of the specimen.

**Tissue Transportation**

Once the ovary was removed, a 3- × 3-mm piece was set aside for permanent sectioning to rule out the presence of cancer cells in the ovary, if applicable. The ovary was then placed in a sterile normal saline or HEPES-buffered Dulbecco’s minimum essential medium (DMEM)-F12 culture media and immediately transported to the IVF laboratory in the adjacent building. In four cases, the ovarian tissue was harvested at an outside center, and then transported to our facility for cryopreservation. In those cases, the transportation of the ovarian tissue was accomplished within less than 1 hour. In two patients, the tissue was cryopreserved and banked elsewhere and was later transported to our Center for ovarian transplantation (6, 7).

**Tissue Processing and Cryopreservation**

In the laboratory, the ovary was transferred to a 10-cm sterile glass dish with a HEPES-buffered wash media that was chilled to 4°C and contained 20% protein. The medium was prepared on site based on Phase-I sequential medium. Immersed in this media, the ovary was bivalved with a scalpel along the hilum and excess ovarian stroma was removed by dissection with iris scissors (11). Any remaining stroma was scraped off with a scalpel, avoiding any cuts to the ovarian cortex. Subsequently the ovarian cortex was cut into 10- × 5-mm pieces of approximately 2-mm thickness. A 5- × 5-mm piece was spared for follicle counts and further histologic analysis. In the meantime, 2-mL cryovials (Nalgene Nunc Inc., Rochester, NY) were filled with 1.5 mL of cryopreservation media, which contained 1.5 M dimethyl sulfoxide (DMSO) and 0.1 M sucrose in addition to the contents of the wash media described previously. No more than two to three pieces were placed in each vial. The containers were...
then agitated on ice for 0.5 hours to enable proper penetration of cryoprotectants into the tissue. Subsequently, the vials were loaded on to a programmable freezer (Planer PLC, United Kingdom) and frozen with a slow freeze protocol as described previously (12). All laboratory processing of the tissue was performed by the same team (either K.O. or O.O.) except for the two cases where the frozen tissues were transferred from elsewhere. In those cases, freezing protocols were identical to ours.

**Tissue Thawing and Transplantation**

The tissue thawing was done as described previously (12). All transplantation cases have already been reported (6–8). Briefly, the frozen vials were first kept at room temperature for 1 minute and then thawed in a sterile water bath set at 30°C followed by transfer of the cortical pieces to thawing media containing gradually decreasing concentrations of cryoprotectant. The transplantation techniques have been described (6, 7) and will not be detailed here. The tissues were transplanted either in the pelvis (orthotopic) (6) or SC in the forearm or lower abdominal area (heterotopic) (7, 8).

**Follow-up Information**

Follow-up was obtained either during the most recent return visit, by phone call from the patient, parents, or if the patient was deceased, from the next of kin.

**RESULTS**

**Subject Characteristics and Indications**

Fifty-nine patients underwent ovarian tissue cryopreservation between May 1999 and March 2008. The mean age (± SE) was 26.7 ± 1.2 years with a range of 4–44 years. Nineteen percent of patients were under the age of 18 years. Sixty-one percent of the patients undergoing ovarian cryopreservation were younger than 30 years (Fig. 1).

In seven patients, ovarian tissue was harvested during laparotomy for another indication. The indications for the laparotomy were stage IC ovarian cancer in three, endometrial cancer in two (stages 1A and 1C endometrioid carcinomas), cervical cancer in one (stage II adenocarcinoma), and cesarean delivery in one woman. The latter patient was diagnosed with breast cancer during pregnancy and underwent ovarian tissue freezing during cesarean delivery.

In all patients at least one ovary was removed for cryopreservation; in one woman, biopsies from the opposite ovary were also cryopreserved. Bilateral oophorectomy and cryopreservation were performed because of Y chromosome mosaicism (13) in one patient, and for breast cancer with BRCA mutations in another patient, for endometrial carcinoma in two cases, and for early ovarian carcinoma in three cases.

The indications for ovarian cryopreservation varied and included noncancer conditions where chemotherapy or radiation treatment was needed (Fig. 2A). The type of treatment that would have been associated with impairment of fertility also varied from chemotherapy, to preconditioning chemotherapy with or without radiation treatment for hematopoietic stem cell transplantation, to radiation and surgery (Fig. 2B). Of those who underwent ovarian tissue cryopreservation before preconditioning chemotherapy with or without total lymphoid radiation, the underlying diseases included hematologic malignancies as well as immunologic, hematologic, and idiopathic noncancerous conditions (Fig. 2A). In the first case of ovarian transplantation, the tissue was cryopreserved elsewhere for the presumed diagnosis of endometriosis. The tissue was transported and transplanted with IRB approval at NY Methodist Hospital (6).

**Risk Assessment**

There were no surgical complications encountered in any of the cases. The average operating time for laparoscopic ovarian tissue harvesting from induction of anesthesia to recovery (mean ± SE) was 83 ± 5 minutes with a range of 43 to 195 minutes. No bleeding occurred, including the three thrombocytopenic patients with platelet counts of 38–46 thousand/mm³, and none required transfusion. In all cases where a laparoscopy was performed, the patient was discharged home the same day. No evidence of cancer cells was found in pathological examination of ovarian tissue in those where a systemic malignancy existed.

**Use of Assisted Reproduction Techniques in Addition to Ovarian Cryopreservation**

Six patients also underwent ovarian stimulation and oocyte retrieval before ovarian tissue harvesting for the purpose of oocyte and embryo cryopreservation. No complications occurred during these procedures. The mean age of these patients was 32 ± 3.4 years (range 22–42 years) with the diagnoses of breast cancer (n = 2), Hodgkin’s disease (n = 1), non-Hodgkin’s lymphoma (n = 1), synovial sarcoma...
A 37-year-old patient, who was diagnosed with breast cancer and received adriamycin and cyclophosphamide (AC) chemotherapy at age 30 years, followed by ovarian freezing at age 33 years, later underwent egg donation and had a live birth.

Long-term Storage and Utilization
The median length of storage at the time of this report was 3.06 ± 0.2 years (0.05–10.5 years). Of those, only three women underwent transplantation with frozen-banked ovarian tissue. The characteristics of patients, diagnosis, types of ovarian transplants, and outcomes are summarized in

FIGURE 2
Diagnoses (A) and treatment types (B) that indicated fertility preservation by ovarian cryopreservation. HSCT = hematopoietic stem cell transplantation.

<table>
<thead>
<tr>
<th>Ovarian transplantation cases</th>
<th>Indications for ovarian cryopreservation</th>
<th>Date of freezing</th>
<th>Date of transplantation</th>
<th>Duration of banking</th>
<th>Age at time of freezing</th>
<th>Age at time of transplantation</th>
<th>Transplantation site</th>
<th>Outcome</th>
<th>Duration of endocrine functioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>Endometriosis</td>
<td>July 1998</td>
<td>February 1999</td>
<td>6 months</td>
<td>28</td>
<td>29</td>
<td>Orthotopic (left pelvic peritoneum)</td>
<td>Endocrine function, ovulation</td>
<td>9 months</td>
</tr>
<tr>
<td>Case 2</td>
<td>Breast cancer (chemotherapy)</td>
<td>May 1997</td>
<td>February 2003</td>
<td>6 years</td>
<td>30</td>
<td>36</td>
<td>Heterotopic (beneath the skin of abdomen)</td>
<td>Embryo development</td>
<td>62 months*</td>
</tr>
<tr>
<td>Case 3</td>
<td>Hodgkin’s lymphoma</td>
<td>March 2002</td>
<td>August 2004</td>
<td>2 years</td>
<td>29</td>
<td>31</td>
<td>Heterotopic (beneath the skin of abdomen)</td>
<td>Spontaneous pregnancy and live birth after transplantation</td>
<td>42 months*</td>
</tr>
</tbody>
</table>

* Continuing ovarian function at the time of this report.

FIGURE 3

A mature oocyte obtained from the Hodgkin’s lymphoma survivor who had undergone subcutaneous heterotopic ovarian transplantation in the lower abdomen.

Table 1. Although the orthotopic ovarian transplantation resulted in ovarian function, which could be confirmed for up to 9 months in one patient, the remaining two transplants to the abdominal SC area continued to function to the date of this report (see Table 1). In one patient who was a breast cancer survivor, a four-cell embryo was generated by aspiration of oocytes from the SC location. In another patient, within 3 months of heterotopic transplantation to the lower abdominal SC area, two spontaneous pregnancies occurred, presumably as a result of renewed ovulatory function in the remaining ovary, resulting in a live birth (8). That patient recently underwent a percutaneous oocyte retrieval that yielded a mature oocyte but failed to fertilize (Fig. 3). Interestingly, this patient has recently conceived spontaneously again, and currently is at the eighth week of gestation. One patient, who had her ovarian tissue cryopreserved during a cesarean delivery before breast cancer chemotherapy, conceived spontaneously while getting ready for an ovarian transplant but had a spontaneous miscarriage in the first trimester.

Follow-up

Fifty-six of 59 patients (94.9 %) have not yet used their ovarian tissue. Of those 50 where follow-up information was available (84.7 %), the reasons for nonutilization were social/personal in 54% (n = 27), still under treatment in 38% (n = 19), and death in 8% (n = 4).

Of the 50 patients who were available for follow-up and after excluding those who were still prepubertal (n = 3), surgically menopausal (n = 6), and deceased (n = 4), 74% developed amenorrhea after chemotherapy.

DISCUSSION

In this article we reported the long-term follow-up and the most comprehensive experience with ovarian cryopreservation and transplantation. Our work indicates that this procedure can be performed in patients with variety of medical conditions and ages without a significant surgical risk. Although most patients who need ovarian tissue cryopreservation and transplantation are oncological patients, indications for ovarian tissue cryopreservation are becoming diverse. Our study also revealed that the experience with ovarian cryopreservation and transplantation is still limited. Because the technology is only a decade old, and because most patients undergoing ovarian cryopreservation are young and many are still undergoing treatment, utilization rate of banked tissue is expectedly low.

Fertility preservation is an individualized choice. In our center, because embryo freezing is the most established method, we offer this option as the first choice to those with steady partners and to single women who prefer to use donor sperm. For single women, we recommend oocyte cryopreservation as hundreds of live births have occurred with this procedure. Ovarian tissue freezing is offered only in those who do not have sufficient time or who is not sexually mature to undergo ovarian stimulation. Embryo and oocyte freezing are relatively more established techniques and do not necessitate removal of an ovary. On the other hand, if successful, ovarian cryopreservation can provide restoration of ovarian function, which cannot be provided by embryo or egg freezing. When ovarian tissue is removed, there is a theoretical possibility that the reproductive life span may be shortened. However, because most candidates for ovarian cryopreservation would have undergone a treatment with a very high probability of premature ovarian failure (POF), as our experience showed, this may not be an actual risk. Nevertheless, as part of this experimental procedure, these pros and cons are discussed with all patients and ultimately they make their own decision with informed consent.

There are still very few reports of live births after ovarian transplantation. In one case, where a relatively less gonadotoxic chemotherapy regimen was used, the patient continued to ovulate from the remaining ovary, and the origin of the spontaneous pregnancy could not be determined with absolute certainty (9, 14, 15). In another patient where the frozen-banked ovarian tissue was implanted in one of the existing but “failed” ovaries, follicle development ensued and a live birth occurred after aspiration of an oocyte and IVF (10). Interestingly, after a heterotopic ovarian transplantation that we performed on a Hodgkin’s lymphoma survivor who became menopausal for 2.5 years after a hematopoietic stem cell transplantation, the patient spontaneously conceived within 4 months of transplantation, concurrently with follicular activity in the ovarian transplant under her abdominal skin. She eventually delivered a healthy girl, who is now 2 years old (8). The same patient has recently conceived again and the pregnancy is currently ongoing. The latter case
clearly illustrates that spontaneous pregnancies can occur, even in those who appear to be in menopause for years, and even after unilateral oophorectomy for ovarian cryopreservation. The latter observation also brings up a new research question as to whether ovarian transplants could play a role in the recovery of the damaged ovary by triggering regeneration of oocytes after chemotherapy (2, 8, 16). However, we cannot conclude from such limited and observational experience whether ovarian transplants might play a role in the recovery of damaged ovaries by triggering regeneration of oocytes after chemotherapy.

The global experience with ovarian cryopreservation and transplantation paints a picture of a highly experimental procedure for which the true success rates are unknown. In the mean time the true value of this procedure has yet to be determined. However, given the fact that many young women, especially those who are prepubertal or who do not have sufficient time to undergo egg retrievals, have no other alternative to preserve their eggs, and given the safety profile of the laparoscopic harvesting procedure presented in this article, research on ovarian cryopreservation should be continued. Although our study suggests that ovarian tissue harvesting is safe in our hands, more extended experience and longer term follow-up will be needed. Because of this, until a critical body of experience is developed with this procedure, it should remain within the realm of specialized research institutions.

REFERENCES