

Ovarian tissue cryopreservation: a committee opinion

The Practice Committee of the American Society for Reproductive Medicine

American Society for Reproductive Medicine, Birmingham, Alabama

Ovarian tissue cryopreservation is an option to preserve reproductive potential in patients who must urgently undergo aggressive chemotherapy and/or radiotherapy or who have other medical conditions requiring treatment that may threaten ovarian function and subsequent fertility. Ovarian tissue cryopreservation may be the only option available to prepubertal girls undergoing such treatments. However, these techniques are still considered to be experimental. This document outlines the current technology, clinical outcomes, and risks of ovarian tissue cryopreservation and recommendations for clinical applications. This document and the document "Mature Oocyte Cryopreservation: A Guideline" published in 2013 (Fertil Steril 2013;99:37-43) replace the document "Ovarian Tissue and Oocyte Cryopreservation" last published in 2008 (Fertil Steril 2008;90:S241-6). (Fertil Steril® 2014; ■ : ■ - ■ . ©2014 by American Society for Reproductive Medicine.)

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REVIEW METHODS

The Committee searched the Medline site up to July 2013 to evaluate the safety and efficacy of ovarian tissue cryopreservation, using the combination of medical subject headings Ovarian tissue cryopreservation and pregnancy, Vitrification, and Whole ovary cryopreservation. Only English-language articles were selected, and the search was restricted to published articles. Review articles were included. The relevance of included articles was assessed by one committee member with subsequent consultation by the committee as a whole.

INDICATIONS FOR OVARIAN TISSUE CRYOPRESERVATION

Ovarian tissue cryopreservation currently is considered to be an experimental technique for fertility preservation. This procedure is an option for patients who require immediate gona-

dotoxic treatment of aggressive malignancies when there is insufficient time to allow the woman to undergo ovulation induction, oocyte retrieval, and cryopreservation of oocytes and/or embryos. Ovarian tissue cryopreservation is the only option available for fertility preservation in young girls who are prepubertal (1-6) or in women who have hormone-sensitive malignancies (7). In addition, women anticipating hematopoietic stem cell transplantation for the treatment of benign hematologic diseases (sickle cell anemia, thalassemia major, aplastic anemia) and women who have autoimmune diseases that have failed to respond to immunosuppressive therapy may elect to prophylactically cryopreserve ovarian tissue (6, 8-12). Other potential indications include fertility preservation in patients with genetic mutations that pose a high risk for premature ovarian failure and who are unable to pursue nonexperimental

fertility preservation approaches. Ovarian tissue cryopreservation should not be offered to women who wish to delay childbearing or to women with benign conditions such as ovarian cysts that are best managed with fertility-sparing surgery.

TECHNIQUES OF OVARIAN TISSUE CRYOPRESERVATION

Ovarian Cortical Tissue

Most oocytes are located within primordial follicles in the ovarian cortex; therefore, obtaining a small volume of cortical tissue potentially enables cryopreservation of large numbers of oocytes. Whenever possible, ovarian tissue should be obtained before a woman initiates treatment. However, for leukemic patients who may harbor cancer cells within the ovarian blood vessels, obtaining ovarian tissue after the first remission and before bone marrow transplant may decrease the risk of transmission from reimplanted thawed tissue (13). The most common method to obtain ovarian tissue is by a laparoscopic approach, although tissue may be obtained by minilaparotomy or at the time of ovarian transposition

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Correspondence: Practice Committee, American Society for Reproductive Medicine, 1209 Montgomery Hwy, Birmingham, Alabama 35216 (E-mail: ASRM@asrm.org).

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surgery. Regardless of the surgical approach, if the anticipated loss of ovarian function after therapy is significant, then removal of more tissue may be appropriate (14, 15). Once the ovarian cortical tissue is obtained, it is transferred to the laboratory on ice and cut into small slivers of tissue that are typically 0.3–2 mm thick and then cryopreserved (4, 16–18). Although this technique has historically been associated with tissue ischemia of the cortical pieces after transplantation, owing to the process of neovascularization, recent studies on the duration of fertility in ovarian cortical grafts show minimal ischemic loss (18–20).

Whole-Ovary Cryopreservation

In patients in whom complete ovarian failure after treatment is anticipated, whole-ovary cryopreservation may be another option. For this indication, the ovary is removed by laparotomy or a laparoscopic approach with a large part of the vascular pedicle left attached (21, 22). Inclusion of a large vascular ovarian pedicle enables the use of special perfusion equipment to introduce cryoprotectant to all cells within the ovary and remove the cryoprotectant at the time of thawing via the ovarian vessels. The large vascular pedicle also facilitates organ transplantation after thawing. This technique has the advantage of promoting early blood flow and oxygenation to the ovary by the anastomosed vascular pedicle, thereby decreasing the risk of ischemia and poor tissue survival seen after transplantation of ovarian cortical fragments because of the process of neovascularization.

Both slow-freezing and vitrification techniques have been used to cryopreserve whole ovaries in animals and humans (21–24). The risks of whole-ovary vitrification include the possibility of fracture of the ovarian pedicle, preventing successful vascular transplantation; fracture of the surface of the ovary as a whole, which then provides an interface for ice crystal formation; inconsistent permeation of the cryoprotectant; and potential for ice crystal formation in the ovarian pedicle or ovary during warming (23). Whereas advantages of whole-ovary cryopreservation include providing an immediate blood supply to the graft, possibly limiting ischemia to the ovary and compromised long-term ovarian function, limitations include the challenges of cryopreservation of the entire ovary, longer surgical time, possibility of ischemia due to thromboembolism within the vascular pedicle, and possibility of reintroduction of cancer cells that may remain in ovarian medullary tissue (e.g., lymphoma) (25).

METHODS OF FREEZING OVARIAN CORTICAL TISSUE

There are two methods of cryopreservation: slow freezing and vitrification. The classic standard for the cryopreservation of ovarian tissue has been slow freezing. However, the vitrification method has gained popularity recently owing to good results obtained with vitrification of oocytes and embryos (26–30). Slow freezing refers to the exposure of the tissue to cryoprotectant and cooling the tissue slowly in a programmable fashion to approximately -140°C , after which time the tissue is put into liquid nitrogen at -196°C

for storage (16, 31–34). Vitrification of ovarian tissue is a rapid method of cryopreservation developed to eliminate the risk of ice crystal formation in ovarian tissue. Vitrification differs from the slow-freezing method in the concentration of the cryoprotectant (high) and the rate of cooling (fast, within minutes) (18, 20, 35–41). Concerns have been raised regarding induction of cellular toxicity and osmotic trauma with the high concentration of cryoprotectants and the safety of vitrification (42). Although no pregnancies have been reported after vitrification of ovarian tissue, outcomes after vitrification of oocytes and embryos appear to be reassuring (30, 43). Further studies are warranted.

In a systematic comparison of vitrification and slow freezing of ovarian tissue followed by tissue culture to assess subsequent oocyte viability, vitrification was demonstrated to have an outcome similar to slow freezing with preservation of the morphologic integrity of the ovarian tissue (38). Although the survival of oocytes was similar between the two methods, granulosa cell survival and the integrity of the stroma were improved with vitrification. Another study evaluating the impact of slow freezing and vitrification compared with fresh oocytes on oocyte survival rates demonstrated higher and similar viability of oocytes in the fresh and vitrified cycles (92%) compared with the slow-freeze cycles (42%), further favoring vitrification (44). Although initial data suggest that vitrification of ovarian tissue may be the favored approach, outcome studies are needed before vitrification replaces slow freezing as the standard method of ovarian tissue cryopreservation.

OVARIAN TISSUE TRANSPLANTATION AND OUTCOMES

Autologous ovarian cortical tissue transplantation has been applied successfully with demonstration of restoration of ovarian function, including achievement of pregnancy and endogenous hormone production. Autotransplantation involves attaching viable cortical ovarian tissue into a pelvic (orthotopic) site or into an extrapelvic (heterotopic) site such as the forearm or the abdominal wall (45–55).

Orthotopic Transplantation of Cortical Tissue

Orthotopic transplantation of ovarian tissue involves transplantation of very thin, <1.0 – 1.5 mm, strips of ovarian tissue that have been successfully thawed into either the medullary portion of the remaining ovary or the peritoneum of the ovarian fossa. Advantages of this technique include the possibility of natural conception as the ovarian tissue is in close proximity to the fallopian tube, and a favorable environment for follicular development. Disadvantages include limitation of the number of fragments transplanted owing to ovarian size and the requirement of an invasive surgical procedure. Resumption of normal ovulatory menstrual cycles has been reported to occur within 4–9 months after transplantation, which is consistent with the time necessary to initiate follicular growth and final maturation (45–47, 56, 57). Tissue ischemia with loss of primordial follicles is a concern,

because these grafts depend on neovascularization of the graft. Studies have shown variability in graft survival and ovarian function, from several months to years, depending on the amount of tissue transplanted and the age of the female when the ovarian tissue was first removed, with the longest graft survival noted to be 7 years (19, 47, 54, 58–62). Ovarian reserve testing has demonstrated that follicle-stimulating hormone (FSH) returns to basal levels following transplantation as estrogen production occurs (62). Anti-müllerian hormone levels remain low after ovarian tissue orthotopic transplantation, presumably reflecting the decreased number of primordial follicles that survive after transplantation (62).

The first case of successful orthotopic autotransplantation of cryopreserved and thawed ovarian tissue was demonstrated in a woman who had previously undergone a bilateral oophorectomy (45). The ovarian tissue was transplanted into peritoneal pockets in the pelvic peritoneum. Ovulation induction with gonadotropins demonstrated follicular development and ovulation, although the patient did not conceive. The first pregnancy was reported in 2004 in a patient who developed ovarian failure after chemotherapy and radiation for clinical stage IV Hodgkin lymphoma (46). Subsequent studies of orthotopic autotransplantation of frozen-thawed ovarian cortical tissue have demonstrated resumption of menses, sonographic documentation of follicular growth, and in some series pregnancies that were conceived without intervention or with in vitro fertilization (IVF) (46, 49, 62).

Pregnancy outcome after orthotopic autotransplantation has been described in a few series, with a recent review reporting 24 births worldwide over the past 10 years with this technique (63). In a review of case reports of pregnancies after orthotopic transplantation (48), 18 pregnancies were reported from 13 patients; however, data regarding pregnancy are confounded by the fact that most of the initial surgical procedures did not involve removal of both ovaries, and the site of ovulation (native ovary versus transplanted ovarian tissue) was not confirmed. A more recent review of data from three programs (which incorporated some of the patients included in the earlier review) reported 11 pregnancies from 60 transplants (18.3%); however, in all pregnancies except one, the patients had native ovarian tissue as well as transplanted tissue (63). Thus in many studies, the success of the orthotopic transplantation procedure is confounded by the possibility that any pregnancy that was conceived could have been the result of ovulation of oocytes from the native ovary and not the transplanted tissue. In addition, the efficiency of this procedure is difficult to determine because it is unclear how many patients have undergone the orthotopic transplantation of thawed ovarian cortex without conceiving, and pregnancy may occur in the setting of primary ovarian insufficiency.

Heterotopic Transplantation of Cortical Tissue

Heterotopic transplantation of cortical ovarian tissue has been performed in the forearm, abdominal wall, and chest wall and has been associated with reports of restoration of ovarian function and follicular development (54, 64). With this option, pregnancy can be achieved only with the use of

oocyte retrieval and IVF using assisted reproductive technologies. Although successful oocyte retrieval and fertilization have been possible, there have been no live births reported with this technique. Potential advantages of heterotopic ovarian tissue transplantation include the beneficial effect of being able to place the grafts in a location that allows for easy follicular monitoring and oocyte retrieval for potential IVF as well as monitoring for recurrent cancer. Disadvantages include compromised viability of the grafts because the heterotopic sites are less likely to undergo neovascularization, a less cosmetically appealing location for the ovary regarding follicular development, and IVF being required for pregnancy to occur (11, 54, 55, 63–65).

WHOLE-OVARY TRANSPLANTATION OUTCOMES

Inadequate neovascularization of transplanted ovarian cortical tissue is thought to be responsible for ischemia and limited survival of the graft. Whole ovarian-tissue transplantation decreases the risk of tissue ischemia because immediate revascularization of the transplant is achieved by utilizing the vascular pedicle (57, 66–72). In humans, fresh whole-ovary transplantation between living-related donor and recipient has been performed successfully (57, 71), with one study demonstrating a live birth (72). There is no report of a successful transplantation of a previously cryopreserved whole ovary in humans. Limitations to the success of whole-ovary transplantation include difficulties with cryopreservation, the small size of the ovarian artery, the inadequacy of the length of the vascular pedicle, and the risk that if the microvascular anastomosis fails, then the survival of the entire ovary is compromised with no option for another attempt at transplantation (23).

SAFETY CONCERNS AND ALTERNATIVE TREATMENTS

The magnitude of risk for reintroducing a malignancy following transplantation of ovarian cortical tissue is currently unknown for most types of cancer. It appears that blood-borne cancers, such as leukemia, carry the highest risk of reintroduction with autotransplantation. A study investigating with the use of immunohistochemistry the presence of residual leukemic cells in frozen-thawed ovarian cortical tissue found that none of the samples had evidence of invasive malignant cells. When 8 of the specimens were subsequently examined with the use of quantitative reverse-transcription polymerase chain reaction (RT-PCR), malignant cells were identified in 6 of the 8 specimens (73). However, when ovarian cortex obtained after treatment and disease remission in patients with leukemia was transplanted into immunodeficient mice, there was no evidence of malignant disease in the ovarian grafts or in any of the other tissues evaluated, despite the fact that 4 of the 7 grafts had malignant cells identified by RT-PCR before transplantation (13). This is in contrast to a study demonstrating the presence of leukemic cells in immunodeficient mice transplanted with ovarian cortex obtained from patients with leukemia who were not in

remission at the time the ovarian cortex was harvested (74). Currently, the magnitude of risk is not known regarding reintroducing malignant cells from grafted tissue, especially when the risk of cancerous involvement of the ovary is limited (3, 75–78). In a systematic review of autotransplantation of ovarian tissue from 289 patients with leukemia, lymphoma, Ewing sarcoma, and colorectal, gastric, breast, endometrial, and cervical cancer, metastases were common in patients who had had leukemia, whereas metastatic disease was less common in most other cancers and was not seen in patients with lymphoma or breast cancer (79). Given the uncertainties regarding transmission of disease, ovarian tissue transplantation is not recommended for patients with blood-borne malignancies, with malignancies that metastasize to the ovary, or with an inherent predisposition to ovarian cancer. However, ovarian tissue transplantation in women with cancers that have a negligible risk of ovarian involvement may be considered for future autotransplantation (80).

Other risks or concerns associated with ovarian tissue transplantation include surgical and anesthetic risks involved in obtaining the tissue and the requirement to provide clear directives regarding disposition of the tissue in the event of the death of the donor. Issues that require further investigation include: optimal cryopreservation technique, length of time the tissue can be cryopreserved, optimal site for autotransplantation, expected survival of transplanted tissue, chance of successful hormonal function related to the graft, chance of spontaneous ovarian function in a residual ovary without any intervention, and chance of pregnancy after a transplant.

ALTERNATIVES TO TRANSPLANTATION

To avoid the risks associated with transplantation of tissue, it would be ideal to be able to isolate and mature oocytes from ovarian tissue for subsequent use in IVF. Reports suggest that intraoperative recovery of immature oocytes from ovarian tissue can be followed by *in vitro* maturation and subsequent cryopreservation of either mature oocytes or embryos (81, 82). However, no live births have been reported from this technique. This approach requires a high degree of collaboration between surgeons and an appropriately trained laboratory staff (83). In addition, basic laboratory research is being conducted to develop methods for isolating and maturing oocytes and follicles of all stages of maturation from previously cryopreserved cortical tissue. To date, this approach has led to live births only in animal models (84, 85).

EXPERIMENTAL NATURE OF OVARIAN TISSUE CRYOPRESERVATION

The American Society for Reproductive Medicine has published specific criteria for the designation of procedures (including tests, treatments, or other interventions) for the diagnosis or treatment of infertility as experimental (86). Procedures will be considered experimental or investigational until the published medical evidence regarding their risks, benefits, and overall safety and efficacy is sufficient to regard them as established medical practice. Relevant medical

evidence can derive only from appropriately designed peer-reviewed published studies performed by several independent investigators, including a description of materials and methods sufficient to assess their scientific validity and to allow independent verification. To date, the published literature describing ovarian tissue cryopreservation does not meet these criteria. The efficiency of these procedures is unknown compared with an untreated control population, because pregnancies may occur in the setting of primary ovarian insufficiency. Similarly, ovarian function may resume after chemotherapy. Furthermore, the safety and efficacy of transplanting ovarian tissue removed from children and adolescents has not been demonstrated. Therefore, the techniques described in this document continue to be considered experimental by the committee.

OTHER CONSIDERATIONS

Currently, the only way to utilize cryopreserved ovarian tissue involves autotransplantation. However, in the future, as discussed above, it may be possible to mature oocytes from cryopreserved ovarian tissue *in vitro* and transfer resulting embryos into a gestational carrier. For this reason, United States Food and Drug Administration infectious disease testing should be considered in patients banking ovarian tissue. See the ASRM Practice Committee document “Recommendations for Gamete and Embryo Donation: A Committee Opinion” for recommended testing (87).

SUMMARY

- Ovarian tissue cryopreservation and transplantation is experimental.
- Ovarian tissue cryopreservation is an option for patients who require immediate gonadotoxic treatment and is the only option available for prepubertal girls.
- Ovarian tissue cryopreservation should not be offered to patients for benign conditions or for the purpose of delaying childbearing.
- Ovarian tissue in humans has been cryopreserved as cortical biopsies or strips or as whole ovaries.
- Ovarian tissue may be transplanted into a pelvic (orthotopic) or extrapelvic (heterotopic) site.
- Pregnancies and live births have been achieved only with orthotopic transplantation of cortical strips; however, data are confounded by the fact that the pregnancy could have resulted from ovulation from a native ovary.
- No pregnancies have been reported to date either from thawed ovarian tissue transplanted into a heterotopic site or as a result of thawed whole ovary transplantation.
- Ovarian tissue transplantation carries a potential risk of reintroducing malignancy.

CONCLUSIONS

- Ovarian tissue cryopreservation should not be offered to patients for benign conditions or for the purpose of delaying childbearing, because it is an experimental procedure.

- Ovarian tissue cryopreservation and subsequent transplant may be offered to carefully selected patients as an experimental protocol.

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Samantha Pfeifer, M.D.; Jeffrey Goldberg, M.D.; Roger Lobo, M.D.; Margareta Pisarska, M.D.; Michael Thomas, M.D.; Eric Widra, M.D.; Jay Sandlow, M.D.; Mark Licht, M.D.; Mitchell Rosen, M.D.; Michael Vernon, Ph.D.; William Catherino, M.D., Ph.D.; Owen Davis, M.D.; Daniel Dumesic, M.D.; Clarisa Gracia, M.D., M.S.C.E.; Randall Odem, M.D.; Kim Thornton, M.D.; Richard Reindollar, M.D.; Robert Rebar, M.D.; and Andrew La Barbera, Ph.D.

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