Ovarian Tissue Cryopreservation

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Abstract

There is a large need for fertility preservation options for cancer survivors, yet at this time, ovarian tissue cryopreservation for fertility preservation remains experimental. Ovarian cortex banking may be offered to patients undergoing gonadotoxic therapy under an approved experimental protocol when ovarian stimulation and in vitro fertilization are not options. Cryopreservation and reimplantation of whole ovaries are areas where more research needs to be performed before it can be offered, even experimentally, to patients desiring fertility preservation. Future work will focus on clarifying patient selection for ovarian tissue cryopreservation. In addition, technical methods of cryopreservation of ovarian tissue should be optimized to enhance follicular survival. Lastly, surgical techniques for revascularization of thawed whole ovaries need to be perfected. The current outlook is hopeful that in the near future, ovarian tissue cryopreservation will be a viable treatment option for fertility preservation.

Keywords

Cryopreservation • Oocytes • Embryos • Ovarian tissue • Fertility preservation gonadotoxic therapy • Ovarian cortex banking

As cancer survival improves, women of reproductive age need options to preserve their fertility. Oncology treatments may involve gonadotoxic levels of chemotherapy or radiotherapy [1–5]. Freezing embryos is a traditional method of fertility preservation. This method has a long track record of success, and women can expect excellent pregnancy rates from cryopreserved embryos, with success rates in the United States ranging from 19 to 36% depending on a woman's age at the time of embryo freezing. However, the disadvantage of this method is that a woman needs a husband, male partner, or donated sperm. For women wishing to preserve fertility but without a male partner or not interested in donor sperm, oocyte cryopreservation is a viable option. Pregnancy rates from cryopreserved oocytes are approaching that seen from cyropreserved embryos in some centers, and it is expected that soon this option will no longer be considered experimental [6].

This chapter will focus on the cryopreservation of ovarian tissue. While this method of fertility preservation is not as well established as embryo or oocyte freezing, it does offer some advantages. Contrary to embryo or oocyte cryopreservation, which requires synchrony with the follicular phase of the menstrual cycle and approximately 10 days of controlled ovarian hyperstimulation, resulting in high serum estradiol levels, ovarian tissue can be extracted and cryopreserved on short notice. It can be offered to women who need timely chemotherapy or who have an estrogen-responsive cancer such as some types of breast cancer. Ovarian tissue cryopreservation offers an advantage here as no pretreatment is

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needed, and a laparoscopy to harvest ovarian tissue can be performed at any point in a woman's menstrual cycle. It can also be offered to children, who are increasingly surviving from childhood malignancies, yet they may lose their fertility as a result of their chemotherapy [7–10]. The ability to preserve future fertility is important for quality of life in cancer survivors. Ovarian tissue cryopreservation may be the only viable option for children with malignancies who need to undergo gonadotoxic treatment.

Cryopreservation of Cortical Strips

The ovarian cortex contains a multitude of primordial and immature follicles. Cryopreservation of the ovarian cortex containing these large numbers of follicles is an emerging area of fertility preservation. The primordial and immature follicles are undifferentiated and are not metabolically active [11, 12]. In addition, the small water content, the high surface to volume ratio, and the absence of the zona pellucida may mean that these follicles are less susceptible to damage during the process of cryopreservation [3]. Ovarian cortical strips can be obtained via a same-day laparoscopy. The ovarian cortex can be harvested during laparoscopy in thin 1–3-mm strips, by removing approximately half the ovary in a block of cortical tissue or by taking small 5-mm biopsies [13, 14]. The use of cautery is avoided during ovarian cortex harvesting. Due to the unpredictable assessment of the risk for gonadotoxicity, some women can be expected to have some ovarian function remaining after chemo or radiotherapy; therefore, it is prudent to leave behind some of the ovarian cortex. With an experienced surgeon, the median operating time is 30 min. The complications from harvesting ovarian cortical strips via laparoscopy are minimal and not different from any other laparoscopic ovarian surgery [15]. However, there are challenges to freezing and reimplanting ovarian cortical strips. These thin strips are subject to ischemic damage both during cryopreservation and retransplantation. There may be poor permeability of the cryoprotectants and resultant freezing damage to the follicles contained within the ovarian cortex. Also, as these strips are frozen without their vascular supply, they rely on neovascularization for their posttransplant survival. Further depletion of follicles in fact occurs when the ovarian graft is reimplanted until a new vascular supply (generally after 5 days to a week) is established to perfuse the graft [16–19].

Ovarian cortical strips can be cryopreserved using both slow freezing and vitrification methods. The methods appear to be comparable in terms of preserving the follicles [20]. Success with cryopreserving ovarian cortical strips and autotransplantation was first demonstrated using animal models. In sheep, fertility was preserved after castration by autotransplantation of cryopreserved strips of ovarian cortex. After ovariectomy and transplantation of frozen–thawed ovarian cortex, FSH and LH levels returned to near normal for 60 weeks and estrous cycles resumed. Yet, a minority of primordial follicles, only 28% survived the process [17]. In the early 1990s, the birth of a lamb resulting from ovulation from a frozen–thawed ovarian cortical graft was reported [11]. Births of lambs were also reported after autotransplantation of cryopreserved hemi-ovaries, but again, only few follicles were seen on histologic examination of the frozen–thawed ovaries [21].

In humans, cryopreservation of ovarian cortical strips as a method of fertility preservation has emerged over the last 10 years. Ovarian cortical strips have been autotransplanted either into the pelvis (orthotopic) or outside the pelvis (heterotopic) [13, 22–27]. Orthotopic autotransplantation involves transplanting the thawed ovarian cortex onto the remaining ovary or into a peritoneal window in the pelvis. The transplantation surgery can be done laparoscopically but has also been done via laparotomy. The first live birth after orthotopic transplantation of cryopreserved ovarian tissue was reported in 2004 [24]. A 25-year-old with stage IV Hodgkin's lymphoma underwent a laparoscopy to biopsy the cortex of the left ovary. Five biopsies were taken and then cryopreserved. The patient received chemotherapy and radiation and then became amenorrheic. Five years later, the ovarian cortex was thawed and then transplanted into a peritoneal window which had been created 7 days earlier to help promote angiogenesis. Eleven months after reimplantation of the ovarian cortex, an intrauterine pregnancy was documented, and the patient went on to deliver a healthy son.

When thawed ovarian cortex is transplanted onto a remaining ovary or on nearby peritoneum, there is not always need for follicular aspiration and assisted reproductive techniques, as the fallopian tube can pick up and transport the ovulated oocyte. However, if the ovarian cortex is transplanted elsewhere, then follicular aspiration and in vitro fertilization are required. Initially, controversy existed because it could not be proven in the cases of orthotopic transplantation that the live birth was not a result of residual ovarian function from the remaining ovary. However, this initial skepticism is subsiding as more live births from the procedure are reported in the literature.

Since this first described successful orthotopic transplantation of frozen–thawed ovarian cortex, many others have been reported. The second successful case involved a 28-year-old with non-Hodgkin's lymphoma and ovarian failure after chemotherapy. She became pregnant after IVF and had a live birth when her cryopreserved ovarian cortex was thawed after 2 years and reimplantated on her ovary [25]. Overall, at least 12 live births have been described after orthotopic transplantation of frozen–thawed ovarian cortex [16, 24–31]. The patients were all in their twenties at the time of ovarian cortex freezing, and the cortex was frozen for a duration of 1–6 years prior to reimplantation. Some of these live births were after spontaneous conception and

others after in vitro fertilization. In 2010, Andersen et al. reported the first women to give birth to a second child after transplantation of frozen–thawed ovarian tissue. She conceived her first child after ovarian cortex reimplantation on her left ovary and delivered a healthy girl in 2007. She spontaneously conceived again that same year and went on to deliver a second healthy girl [32].

Heterotopic transplantation of ovarian cortex has also been described. In 2004, Oktay et al. described cryopreserving ovarian tissue from a 30-year-old woman with breast cancer prior to chemotherapy-induced menopause [23]. The cortex was then transplanted to the skin beneath her abdominal skin. After undergoing eight cycles of in vitro fertilization, one oocyte, out of 20 retrieved, fertilized normally and developed into a 4-cell embryo.

In 2006, a case was reported of a woman diagnosed with Hodgkin's lymphoma who had frozen–thawed ovarian tissue transplanted to a subperitoneal pocket on the lower abdominal wall. Twice an oocyte was retrieved from this location and fertilized by ICSI. After one of the embryo transfers, a biochemical pregnancy occurred [33]. Heterotopic transplantation of ovarian cortex tissue to other sites such as the forearm has also been described [13, 22, 23]. In monkeys, a successful heterotopic transplantation of fresh ovarian tissue, which has led to the birth of a healthy female after oocyte production, fertilization and transfer to a surrogate mother has been reported [34]. A live birth after heterotopic ovarian cortex transplantation has not yet been reported in humans, so this approach to ovarian tissue cryopreservation is nowadays rarely recommended.

Risks

One major concern of cryopreserving tissue from patients with a malignancy and then reimplanting this tissue is the risk of metastasis [35]. In one Danish study, in which females with leukemia had ovarian cortex cryopreservation, 6 of 8 patients had PCR evidence of the leukemic cells in the ovarian tissue [36]. Molecular markers of malignant cells allow detection of small numbers of cells by PCR which cannot be detected by histologic examination.

Another group examined ovarian cortex biopsies of a patient with CML and did not find evidence of malignant cells by routine histologic methods, but again, identified some evidence of malignant cells by PCR. It is unclear whether this PCR evidence of malignant cells came from the ovarian biopsies or from contaminating blood [35]. The viability of these malignant cells is unknown.

A study examined the presence of malignant cells in ovarian cortex of women with CML and ALL and found malignant cells present by PCR in 2 out of 6 patients with CML and 7 out of 10 patients with ALL. Further, when the ovarian cortex tissue of patients with ALL was xenografted into immunodeficient mice, the mice developed intraperitoneal leukemic masses [37]. These findings suggest that in patients with leukemia, reimplantation of ovarian cortex should not be recommended. Other malignancies seen in the reproductive years with a relatively high risk of ovarian metastasis are Burkitt's lymphoma and neuroblastoma. Reimplanting ovarian cortex may not be recommended in these malignancies [38, 39].

Advanced-stage breast cancer (stages III and IV) can metastasize to the ovary, and in patients with known metastatic breast cancer, it may be prudent not to cryopreserve and then reimplant cortical strips [14]. In contrast, more than 10 women with Hodgkin's lymphoma have received reimplanted ovarian cortex tissue, and no relapses have been documented so far [40–42]. All ovarian cortex tissue should be examined for malignant cells or minimal residual disease prior to being reimplanted [3, 43].

As ovarian tissue cryopreservation and reimplantation techniques are fairly new and not in widespread use as of this publication. The true long-term risk of metastasis in patients with malignancy who had ovarian cortex reimplanted remains to be seen.

Whole Ovary

Cryopreservation of ovarian cortical strips and then reimplantation lead to follicular loss secondary to ischemia. Transplantation of a whole ovary may result in less follicular loss as vascular supply to the tissue in theory can be more quickly established, decreasing follicular loss secondary to ischemia. The challenges of whole ovary cryopreservation include optimizing methods for cryopreservation of large organs such as the ovary, which can be difficult as cryoprotectants do not diffuse well into whole organs and intravascular ice formation can cause vascular injury. Also, the technical aspect of harvesting and reimplanting a whole ovary requires a skilled surgeon to preserve a long and intact vascular pedicle at the time of harvesting and then perform the vascular reanastomosis at the time of reimplantation.

Whole ovary autotransplantation was first described using fresh ovaries in various animal models, including rats, sheep, and monkeys. Some authors reported on sheep ovaries, which were autotransplanted into the abdominal wall with microsurgical vascular anastomosis of the ovarian to the inferior epigastric vessels. After 7 days, the ovaries were removed and noted to have surviving follicles [44].

In a rodent model, successful transplantation of ovaries, fallopian tubes, and the upper segment of the uterus en bloc after cryopreservation was reported in 2002. In four out of seven attempts at transplantation, the rat ovaries survived 60 days or more and one pregnancy resulted [45]. Recently, adult female sheep have become a standard model to study intact ovary cryopreservation [46, 47]. Arav et al. described

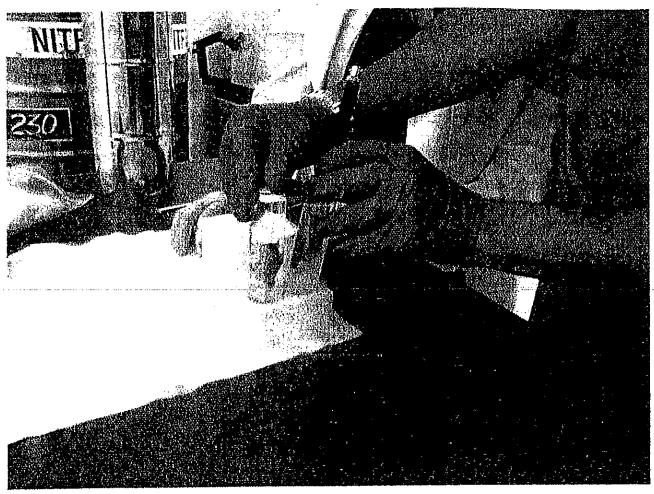

Fig. 61.1 Seeding of whole ovary in the glass tube prior to being loaded in the Multi-Thermal-Gradient device for cryopreservation

transplantation of frozen–thawed intact ovaries in eight sheep by artery and vein anastomosis to the contralateral ovarian artery and vein. From 24 to 36 months after the ovary was reimplanted, progesterone activity was detected in three sheep. Oocyte retrieval was successful in two sheep, and in embryonic development up to the 8-cell stage was noted [48]. Bedaiwy et al. described restoration of ovarian function in frozen–thawed sheep ovaries reimplanted with microvascular anastomosis. Yet, 8 of 11 ovaries failed due to thrombosis at the pedicle site [49]. In 2006, a live-born lamb was reported after orthotopic microvascular reanastomosis of a whole cryopreserved ovary [50]. Whole ovary cryopreservation in the sheep has been attempted through both slow-cooling and vitrification methodologies [47, 51–53]. Recently, ovarian function of cryopreserved and transplanted whole sheep ovaries has been demonstrated 6 years after transplantation. This is the longest reported ovarian function of frozen–thawed whole ovaries [54].

In humans, the first report of cryopreservation and then thawing of a whole ovary was described by Martinez-Madrid et al. in 2004. They found that the percentage of live follicles was 99.4% in fresh tissue, 98.1% after cryoprotectant exposure, and 75.1% after thawing, and they also reported high survival rates of stromal cells and small vessels after thawing [55]. Bedaiwy et al. have recently described that successful cryopreservation of the human ovary in two premenopausal women with overall viability of the primordial follicles was 75 and 78% in intact cryopreserved–thawed ovaries [49]. Further, Patrizio et al. reported on successful whole human ovary cryopreservation with the vascular pedicle utilizing a Multi-Thermal-Gradient device and a slow-cooling, rapid-thawing protocol (Fig. 61.1) [56]. The ovaries were thawed after cryopreservation for 2–4 days, and the frozen–thawed ovary was histologically similar to the fresh contralateral ovary used as a control and modest increase in markers of apoptosis. In three cases, the fallopian tube was cryopreserved along with the whole ovary, and after thawing, the histologic architecture was intact [57].

Table 61.1 A comparison of cryopreservation of cortical strips vs. whole ovaries

	Cortical strip	Whole ovary
Laparoscopic harvesting possible	Yes	Yes
Preserves ovarian stroma	No	Yes
Loss of follicles after cryopreservation	Yes	Yes
Loss of follicles due to ischemia	Yes	Yes
Microvascular anastomosis possible[a]	No	Yes
Short-term endocrine function	Yes	Yes
Long-term endocrine function	No	Yes

Adapted from Bromer and Patrizio [58], with permission
[a]In the event of anastomosis failure, the whole ovary will be lost. Cortical strips offer an advantage here because multiple strips are harvested and transplanted. If the loss of one cortical strip occurs, the others may remain viable

At the time of this writing, however, no cases of reimplanting a frozen–thawed whole ovary resulting in a live birth have been reported in humans. It is possible that the risks of whole ovary transplantation are greater than with ovarian cortical strips, as transplantation of a whole ovary may result in a higher risk of metastasis. Also, when transplanting organs such as a whole ovary, if the vascular anastomosis fails, then the whole ovary is lost. This compares to the transplantation of cortical strips which can be reimplanted in batches so that if the initial procedure fails, it can be repeated with the remaining cortical strips. See Table 61.1 for a comparison of the cryopreservation of whole ovaries vs. cortical strips.

Conclusions

There is a large need for fertility preservation options for cancer survivors, yet at this time, ovarian tissue cryopreservation for fertility preservation remains experimental. Ovarian cortex banking may be offered to patients undergoing gonadotoxic therapy under an approved experimental protocol when ovarian stimulation and in vitro fertilization is not an option. Cryopreservation and reimplantation of whole ovaries are areas where more research needs to be performed before it can be offered, even experimentally, to patients desiring fertility preservation. Future work will focus on clarifying patient selection for ovarian tissue cryopreservation. In addition, technical methods of cryopreservation of ovarian tissue should be optimized to enhance follicular survival. Lastly, surgical techniques for revascularization of thawed whole ovaries need to be perfected. The current outlook is hopeful that in the near future, ovarian tissue cryopreservation will be a viable treatment option for fertility preservation.