Prospective solutions to ovarian reserve damage during the ovarian tissue cryopreservation and transplantation procedure

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Birth rates continue to decline as more women experience fertility issues. Assisted reproductive technologies are available for patients seeking fertility treatment, including cryopreservation techniques. Cryopreservation can be performed on gametes, embryos, or gonadal tissue and can be used for patients who desire to delay in vitro fertilization treatment. This review focuses on ovarian tissue cryopreservation, the freezing of ovarian cortex containing immature follicles. Ovarian tissue cryopreservation is the only available treatment for the restoration of ovarian function in patients who undergo gonadotoxic treatments, and its wide adoption has led to its recent designation as "no longer experimental" by the American Society for Reproductive Medicine. Ovarian tissue cryopreservation and subsequent transplantation can restore native endocrine function and can support the possibility of pregnancy and live birth for the patient. Importantly, there are multiple steps in the procedure that put the ovarian reserve at risk of damage. The graft is highly susceptible to ischemic reperfusion injury and mass primordial follicle growth activation, resulting in a "burnout" phenomenon. In this review, we summarize current efforts to combat the loss of primordial follicles in grafts through improvements in freeze and thaw protocols, transplantation techniques, and pharmacologic adjuvant treatments. We conducted a review of the literature, with emphasis on emergent research in the last 5 years. Regarding freeze and thaw protocols, we discuss the widely accepted slow freezing approach and newer vitrification protocols. Discussion of improved transplantation techniques includes consideration of the transplantation location of the ovarian tissue and the importance of graft sites in promoting neovascularization. Finally, we discuss pharmacologic treatments being studied to improve tissue performance postgraft. Of note, there is significant research into the efficacy of adjuvants used to reduce ischemic injury, improve neovascularization, and inhibit hyperactivation of primordial follicle growth activations. Although the "experimental" label has been removed from ovarian tissue cryopreservation and subsequent transplantation, there is a significant need for further research to better understand sources of ovarian reserve damage to improve outcomes. Future research directions are provided as we consider how to reach the most hopeful results for women globally. (Fertil Steril® 2024;122:565–73. ©2024 by American Society for Reproductive Medicine.)

Key Words: Ovarian tissue cryopreservation, transplant, freeze and thaw, vitrification, neovascularization

There are an estimated 18 million cancer survivors in the U.S., a number projected to grow to 26 million by 2040 (1). With modern medical advances, 85% of children diagnosed with cancer survive to adulthood, and it is estimated that 76% of survivors want to have children in the future. Although women receiving gonadotoxic therapy may have the option to freeze oocytes or embryos before treatment, for many patients, ovarian tissue cryopreservation (OTC) is the only available option (2). For example, OTC can be used to assist pre- or peripubertal female cancer patients who are not candidates for ovarian stimulation or would not tolerate the procedure itself. Ovarian tissue cryopreservation can be also an option for women who require immediate chemotherapy and do not have time to undergo ovarian stimulation, a process that can take 2–3 weeks. Indeed, in 2019, the Practice Committee of the American Society for Reproductive Medicine removed the "experimental" label from OTC, stating that "Ovarian tissue banking is an

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acceptable fertility-preservation technique and…is the only method to preserve fertility for prepubertal girls since ovarian stimulation and in vitro fertilization treatment are not options" (3–5). Furthermore, OTC with subsequent graft transplant (OTC/T) is increasingly being explored as a technique to delay menopause, a condition for which few treatments are available–although, studies for this indication are limited (6).

Ovarian tissue cryopreservation involves the surgical removal and cryopreservation of either a section of the ovarian cortex or the whole ovary. The ovarian tissue, which ideally contains thousands of primordial follicles (PFs), is cryopreserved, remaining safely frozen and protected from the effects of gonadotoxic therapy indefinitely until pregnancy is desired. At that time, the thawed tissue can be grafted back into the patient, the goal being that surviving ovarian follicles will reinitiate growth patterns and restore the possibility of conception. Ovarian tissue cryopreservation success rates have improved markedly over the last decade: as of 2023, >130 infants have been born worldwide after OTC. A 2022 meta-analysis reported a pooled live birth rate for frozen transplants of 28% (95% confidence interval [CI]: 32%–43%) (7). In another case series of 20 patients undergoing ovarian tissue autotransplantation after OTC, 53% of patients conceived, and 32% had at least one birth; one patient conceived 4 times (8). Ovarian function is restored in up to 95% of OTC/T cases (8, 9). Markers of endocrine function after graft transplant include serum estradiol (E2) and follicle-stimulating hormone levels (10–12). Khattak et al. (7) reported a mean posttransplant E2 of 522.4 pmol/L (95% CI: 315.4–729), a follicle-stimulating hormone level of 14.1 IU/L (95% CI: 10.9–17.3), and a median duration of graft function of 2.5 years.

Unfortunately, massive loss of ovarian follicles during tissue freezing, thawing, and transplant remains a significant limiting factor in OTC success. Poor PF survival limits the duration of function of ovarian tissue grafts and the number of mature oocytes that can be produced by each graft. Furthermore, a consistent "standard of care" protocol for OTC does not yet exist. Thus, although OTC represents an attractive fertility-preservation option for specific individuals, improvements in OTC techniques are urgently needed. In this review, we provide a critical assessment of current OTC techniques and discuss strategies to maximize outcomes for patients relying on OTC/T.

MATERIALS AND METHODS

We searched the PubMed database for studies of OTC and transplantation published from January 2019 to June 2024, including meta-analyses, systematic reviews, and observational studies. A total of 27 articles were identified, including 15 experimental studies, 4 systematic reviews, 1 meta-analysis, and 7 practice guidelines. We manually searched references and found an additional 83 relevant publications that were published outside of the 2019–2024 timeframe.

BACKGROUND

Understanding the basics of primordial follicle activation

Primordial follicles are the most immature state of follicles in the ovary, each consisting of a primary oocyte surrounded by a single layer of squamous granulosa cells (13). Primordial follicles are located in the outer cortex of the ovary, a thin, dense, fibrous layer that serves as a PF reservoir and site of the initial stages of follicle development (14, 15). The ovarian cortex interacts closely with the inner medulla, which is highly vascularized and contains connective tissue, lymphatic ducts, and nerves. The medulla supplies blood, nutrients, and hormonal signals to the cortex, supporting follicular development and overall ovarian function.

Primordial follicle growth activation (PFGA) is a process in which individual PFs leave their dormancy, and begin the process of folliculogenesis. Loss of PFs from the "ovarian reserve" controls the length of the female fertile window. The pattern of PFGA and loss during adult life approximates exponential decay (16). Once activated, follicles grow and develop through primary, secondary, and antral follicle stages and eventually peri-ovulatory follicles capable of undergoing ovulation. Of the PFs present early in postnatal life (up to 1 million), most are committed to death after growth initiation, and only a small fraction survive to ovulate. Several signaling pathways have been shown to dictate the activation process, including the c-Kit and Kit ligand pathway and the phosphatase tensin homologue, phosphoinositide 3-kinases, protein kinase pathway, both of which are crucial for oocyte survival and growth (Fig. 1). Growth factors such as oocyte-secreted growth differentiation factor and bone morphogenetic protein 15 also play significant roles in coordinating the proliferation and function of surrounding granulosa cells. How the process is regulated physiologically to balance the preservation of the ovarian reserve and a steady supply of growing follicles is an area of active investigation (16, 18).

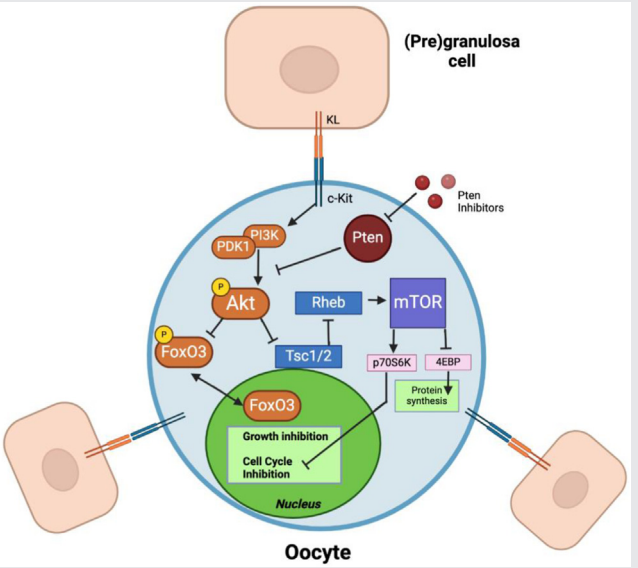
For OTC to be successful, the removal, freezing, and grafting of ovarian tissue must result in the normal development of PFs. That is, PFs must not only survive but must then engage in development in a way that approximates the pattern of PF loss and restores the correct stages of development through ovulation. This restoration of ovarian function at the level of follicle behavior can be seen in the many cases where ovarian function resumes and natural conception is achieved post-OTC/T (7–9, 14, 19–23).

How do the individual steps of OTC affect PF numbers and graft performance?

A brief overview of the OTC procedure. Ovarian tissue cryopreservation involves surgical and cryopreservation processes that can impact the survival and functionality of PFs. In brief, either a piece of ovarian tissue or an entire ovary is removed using laparoscopy (23, 24). After removal, medullary tissue is separated from the cortex; the cortical tissue is sectioned into small pieces for freezing (23, 25). When the patient is ready to attempt to conceive, the cryopreserved pieces of ovarian tissues are then thawed and transplanted back into the patient. The thawed ovarian tissue may be surgically placed on the remaining ovary or can be transplanted intramuscularly, subcutaneously, or onto the pelvic sidewall (7).

The most widely performed protocol for cryopreservation is slow freezing, which involves slowly cooling the tissue to −140 °C with storage in liquid nitrogen. This protocol

FIGURE 1



Signaling pathways that regulate primordial follicle growth activation (PFGA). This figure (adapted from [14, 17]) depicts a primordial follicle (PF), including oocyte (blue, central) and pregranulosa (peach, surrounding) cells. The signaling cascade that modulates PFGA involves the Kit Ligand (KL) and c-Kit receptor tyrosine kinase proteins. Through signaling of phosphoinositide 3-kinases (PI3K)/3-phosphoinositide dependent protein kinase-1 (PDK1), phosphorylation of protein kinase B (AKT) inhibits transcription factor forkhead box O3 (FoxO3) nuclear transit, and thereby negatively regulates cell growth and cell cycle progression. Through a related signaling cascade, phosphorylated AKT can inhibit the activation of the mammalian target of rapamycin (mTOR), which leads to decreased protein synthesis. Both pathways can be modulated by the upstream action of phosphatase tensin homologue (PTEN). From Kallen et al. [14] and Adhikari and Liu [17] Reprinted by permission of the publisher.

Mercier. OTC/T procedure improvements. Fertil Steril 2024.

has proved very effective, although it does pose the risk of ice crystal formation, which could mechanically damage the cells in the tissue (26, 27). Ovarian tissue is frozen with cryoprotectant agents (CPAs), which protect the integrity of cell membranes and intracellular environments and prevent tissue injury from freezing damage during cryopreservation. CPAs can be classified as either "penetrating" or "nonpenetrating." Penetrating cryoprotectants act as solvents, reducing the solute concentration in the remaining water fraction inside the cell until cooling is achieved. Penetrating CPAs include dimethylsulfoxide, 1,2-propanediol, ethylene glycol, and glycerol. A concern with penetrating CPAs is the possibility of cell damage from cytotoxins in the cryoprotectant itself. Nonpermeating CPAs, conversely, are large molecules that do not penetrate the plasma membrane and remain in the extracellular compartment. Nonpenetrating cryoprotectants include sugar-based substances such as sucrose, trehalose, and raffinose. Nonpenetrating CPAs, which are typically less cytotoxic than penetrating CPAS, dissuade ice crystal formation by forming hydrogen bonds with water molecules and can allow for lower concentrations of permeating CPAs to be used without compromising vitrification properties. Some literature suggests that nonpenetrating CPAs with limited cytotoxicity can effectively replace penetrating CPAs (23).

Contributing factors to PF loss. Ensuring an adequate PF density in the cryopreserved sample is of utmost importance; in a study of 60 patients, reinitiation of menstrual cycles was achieved in 93% of cases (52 patients) when transplants contained detectable PFs, whereas grafts lacking PFs did not support cycle reinitiation (21, 22). Factors including patient age, smoking status, prior exposure to gonadotoxic chemotherapy or radiation, and the amount of cortex removed all determine the PF endowment available for cryopreservation (23).

Removal of ovarian tissue from the body results in immediate tissue ischemia until the graft is cryopreserved. However, a study of the effects of warm ischemic time before cryopreservation on sow ovaries found that an ischemic time of <30 minutes before exposure to cryoprotectants did not significantly decrease PF numbers (28). Major loss of follicles is also not thought to occur during the freezing and thawing process itself; a study of PF survival during OTC and tissue grafting in immunodeficient mice found that only 7% were lost during the cryopreservation process (29). Conversely, significant loss does occur after the transplant of the graft; in the same study, only 35% of oocytes survived the initial grafting, presumably because of damage occurring as a result of ischemia. In another study, a transplant of whole mouse ovaries was performed either immediately or after cryopreservation; histologic sections evaluated 2 weeks later showed a 42% decrease in PF counts after fresh transplant, although freezing and thawing of the tissue increased PF loss by only an additional 9% (30). These data further suggest that most PF loss occurs as a result of transplantation rather than the cryopreservation and thawing steps.

After the transplant, the ongoing survival of the graft depends on the resolution of tissue ischemia by

neovascularization. For the first 3–5 days after transplantation, the graft is supported primarily by diffusion of nutrients and oxygen from surrounding tissues (31). Significant reperfusion does not begin until day 5 of posttransplantation healing (32), and it is not until up to 10 days after transplantation that the graft is fully revascularized (33–35). Although angiogenesis occurs in the transplanted graft, inflammatory mediators, free radicals, and reactive oxygen species (ROS) can compromise the integrity of the graft (36–38). Furthermore, there is differential survival of PFs and other "compartments" of the ovary during revascularization. The ovarian cortex can survive ischemic conditions for at least 3 hours, and PFs can survive for 4 hours when stored on ice. Ovarian stroma, however, is much more sensitive to hypoxic conditions (37, 39, 40). In mouse models, approximately 25% of PFs are destroyed during transplantation (37, 41, 42), whereas the number of growing follicles is depleted by 60%–95% (29, 33, 37, 43–45). This difference in survival between PFs and growing follicles is attributed to the low metabolic rate of PFs and their location in the ovarian cortex, which is a more hypoxic environment than the ovarian medullary layer (36, 40, 46–48).

In addition to PFs, the graft contains larger, developing follicles that are susceptible to injury when the graft is prepared, either during mechanical preparation or during cryopreservation (29, 36, 43–45). Growing follicles produce antimüllerian hormone (AMH), a critical "gatekeeper" hormone that inhibits PF activation and preserves the dormant follicle pool; mouse models of AMH loss exhibit accelerated activation of dormant PFs (36, 49, 50). A large body of evidence suggests that a phenomenon of "mass activation" of PFs occurs after transplantation of frozen-thawed ovarian tissue and significantly contributes to PF loss after grafting. In a study of marmoset, bovine, and human ovarian cortical slices transplanted into mice, a significant increase in the number of growing follicles and increased proliferation of granulosa cells in posttransplant tissue was observed. This finding corresponded to a statistically significant loss of PFs 3 days posttransplantation in human ovarian graft tissue (51). Other studies of human ovarian tissue grafts found significant proliferation in transitional and early-growing follicles, with 80%–95% of growing follicles actively proliferating in transplanted graft tissues as compared to only 18%–40% in nontransplanted tissue (49, 52).

HOW CAN WE IMPROVE OTC?

For OTC to result in successful conception and live birth, there must be a sufficient number of surviving PFs in the transplanted graft. The overarching goal is to accurately mimic normal ovarian physiology in the absence of OTC. Although it is difficult to precisely measure the relative contribution of cell death and potentially excess activation (and subsequent death), when mass activation does occur, PFs may be so greatly exhausted that too few are available to support ovarian function (53, 54). In the next section, we will consider individual steps in the OTC process as potential targets to improve PF survival after transplant (Fig. 2).

Changes in freeze and thaw protocols

An alternative to the slow freezing of ovarian tissue, known as vitrification, has demonstrated validity in a few clinical settings (23, 55). Vitrification, or "ultrarapid freezing," involves immediate freezing of the tissue with high concentrations of cryoprotectant (27). The increased viscosity of the cryoprotectant solution used for vitrification results in a lowered melting temperature and thus a lower risk of ice crystal formation that can damage tissues (56). However, because of the high concentrations of cryoprotectants required for immediate tissue freezing, there is the risk of chemical toxicity, osmotic damage, and thermomechanical stress (23). Vitrification is the first-line cryopreservation technique for freezing embryos and oocytes, but there is substantially less published data regarding the efficacy of vitrification for ovarian tissue. In 2017, a meta-analysis of 14 studies comparing vitrification and slow freezing found significantly reduced DNA damage in PFs and better preserved stromal cells when frozen with vitrification, although there was not a significant difference in the number of surviving PFs between the 2 techniques (57). Although there is no consensus regarding the optimal standard procedure for freezing, vitrification appears promising in its results thus far.

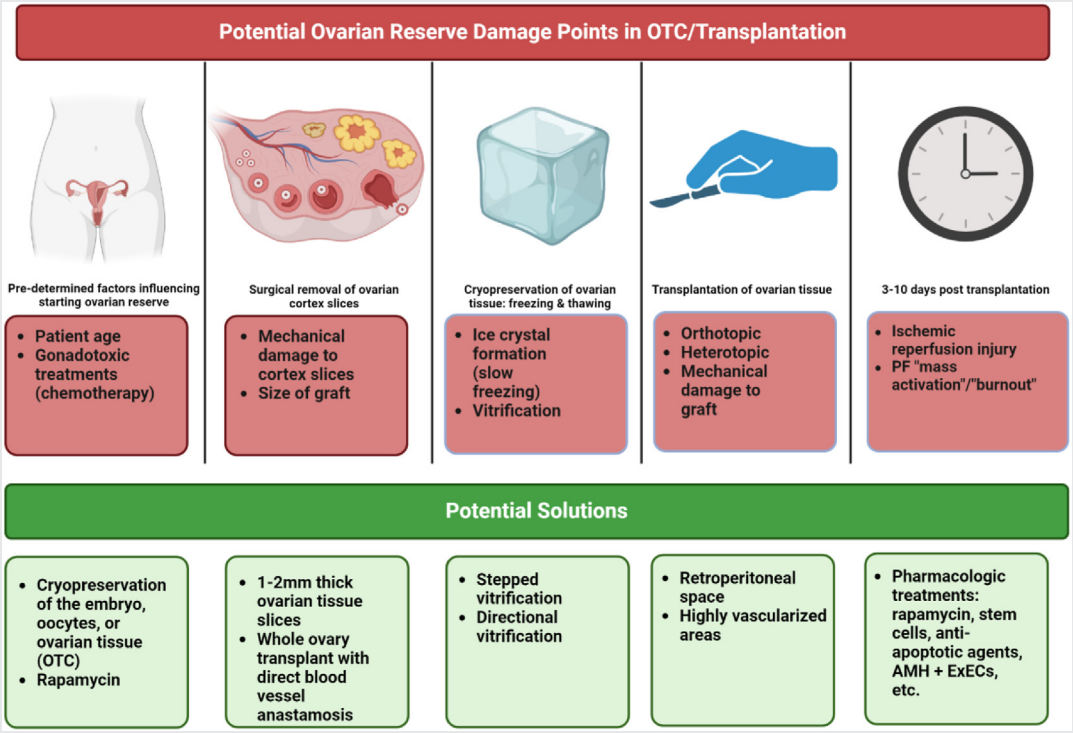
A recent hybrid version of both the slow freezing and vitrification techniques has been reported, termed "stepped vitrification." This protocol involves utilizing both stepwise concentrations of cryoprotectant and decreases in temperature (58). Stepped vitrification is advantageous because it may eliminate the threat of ice crystals possible in current slow freezing protocols, while mitigating the potential for gonadotoxic damage from high cryoprotectant concentrations presented by traditional vitrification protocols (56, 58). The stepped vitrification protocol is encouraging in concept and preliminary results but requires more validation of tissue viability posttransplantation (58). Similar in concept, a "directional vitrification" protocol allows for stepwise temperature control at a constant velocity to protect against the formation of ice crystals. There is ample data suggesting this is a reliable cryopreservation method for both cortical ovary slices and whole ovaries in sheep (59–62). Ultimately, further research is needed to determine whether traditional, stepped, or directional vitrification should replace slow freezing as the standard of care in human OTC protocols (27, 63).

Technical aspects of ovarian tissue transplant

Site of transplant. The current method of transplantation of ovarian tissue is highly patient-specific. Ovarian tissue can be transplanted orthotopically, such as onto the ovary or pelvic sidewall. An orthotopic transplant permits spontaneous conception, although this possibility may be limited if the patient has significant scarring or vital reproductive organ loss (64). Alternatively, the graft may be transplanted heterotopically, such as into a subcutaneous location on the forearm, an intramuscular site within the abdominal wall, or a retroperitoneal or preperitoneal site (60–62, 65).

Whatever location is chosen, ensuring an adequate supply of blood and oxygen to transplanted tissue is crucial for

FIGURE 2



Potential ovarian reserve damage points in the ovarian tissue cryopreservation (OTC) and subsequent transplantation (OTC/T) procedure. This figure (adapted from [36]) depicts the potential points in OTC/T in which the ovarian reserve may be diminished. Considerations related to each step of the procedure are outlined in the image, along with a corresponding proposed solution(s). Each approach is discussed in greater detail as follows. From Roness et al. [36] Reprinted by permission of the publisher. AMH = antimüllerian hormone; ExEC = exogenous endothelial cell.

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the success of the procedure (66). Hypoxia and ischemia currently pose a large challenge for the first 3–5 days post-grafting in animal models (31, 67). The site of the transplant may dictate the cells' recovery from hypoxia and ischemia. Angiogenic factors, such as calcium ions (Ca^{2+}) and transient receptor protein channels, are abundant in the retroperitoneal organs and can influence neovascularization at the chosen transplant site (68, 69). Transplanting ovarian tissue into the retroperitoneum may also allow for the coadministration of treatments to support PF survival, such as angiogenic factors, stem cells, or growth factors (69, 70).

Graft size. Currently, the process of obtaining ovarian tissue for freezing involves trimming the ovarian cortex from the ovary. After the removal of a cortical strip, the tissue is cut into smaller sizes for cryopreservation, typically with dimensions ranging between 4–10 mm in length, 4–10 mm in width, and 1–2 mm in thickness (23, 71–76). The biomechanical properties (e.g., stiffness) of tissue depend on thickness and size and may influence follicle survival and intrafollicle signaling (77–79). A systematic review of OTC/T suggests the best outcomes occur with 1–2 mm thick ovarian tissue samples; this size minimizes ice crystal formation and ischemic time (80–82). In studies that utilized thinner strips of ovarian tissue with the goal of reducing the distance for growth factors and other molecules, follicle activation and a lack of revascularization were observed (36, 83). Some evidence suggests that cryopreserving tissue pieces that contain both the ovarian cortex and some of the medullary layer can promote neovascularization posttransplantation (84). The current practice in adolescents and adults is to remove the medullary layer from the cortex and only cryopreserve the cortex (76).

Transplanting a cryopreserved whole ovary has theoretical benefits by allowing the establishment of vascular anastomosis, which would allow the graft to remain stable for a longer period of time than ovarian cortical strips. A systematic review in 2020 examined 53 experiments of whole ovary transplantations and identified 3 major challenges with this method: the surgical expertise required for both retrieval of the whole ovary and its vascular pedicle, subsequent vascular anastomosis during transplantation, and applying the cryopreservation procedures to the whole ovary. There have been a limited number of attempts at fresh whole ovary transplant in humans, and none involving transplant of a previously cryopreserved ovary (85). When transplanted in sheep, there does not appear to be a benefit to cryopreserving the entire ovary, as vascular and stromal damage were still present (66, 86, 87).

The use of adjuvants to improve PF survival

Adjuvants to reduce graft ischemic injury. Because up to two-thirds of the grafted tissue may be damaged because of ischemia (11, 32, 33), the use of pharmacologic treatments aimed at reducing ischemic reperfusion injury postovarian tissue transplant is of particular interest. Mahmoodi et al. (10, 88) reported that mice treated with erythropoietin (EPO) or N-acetylcysteine daily, beginning 1 day before ovarian tissue transplant until 7 days posttransplant, exhibited decreased apoptosis and increased PF survival in transplanted grafts. In EPO-treated groups, there was also an increase in the mean total volume of the medulla and cortical tissue, an increased number of primordial, primary, preantral, antral, and total follicles, and increased serum estradiol levels (10, 36, 88). Another study of N-acetylcysteine, administered individually or coadministered with E2 to ovarian tissue graft recipient mice, demonstrated decreased oxidative damage, increased angiogenesis, and an increased number of primordial, preantral, and antral follicles after both treatments (89). Verapamil, a calcium channel blocker, has been also shown to increase graft PF survival; the mechanism for this effect is not known (36, 90).

Adjuvants to improve angiogenesis and neovascularization. There is evidence supporting the idea that cotransplanting ovarian tissue grafts with mesenchymal stem cells can increase neovascularization in transplanted tissues. Mesenchymal stem cells are thought to protect the development of PFs by decreasing apoptosis and increasing expression of CD31 (a biomarker for platelet endothelial cell adhesion molecule), CD34 (a biomarker for hematopoietic stem cells), and vascular endothelial growth factor A (91). Similar results have been observed after cotransplantation of high concentrations of adipose tissue-derived stem cells (67, 92). Potential pharmacologic treatments to improve angiogenesis posttransplant include vascular endothelial growth factor, which increased the number of PFs after transplant in a mouse ovarian transplant model (36, 93), and fibroblast growth factors such as basic fibroblast growth factor, which have been shown to increase graft PF counts in both mouse and human models (36, 94, 95).

Adjuvants to prevent PFGA hyperactivation. The offset of PF activation and subsequent mass death of cells is an improvement point of focus to improve the OTC procedure. The mammalian target of rapamycin (mTOR) is a protein kinase that has critical biologic functions, including the modulation of signaling cascades regulating PFGA (Fig. 1) (96). Thus, targeting mTOR activity represents a promising approach to preventing premature PFGA in ovarian grafts. Indeed, evidence suggests that administration of the mTOR inhibitor rapamycin protects PFs after transplantation and promotes their dormancy (67, 92). Rapamycin is an approved immunosuppressive drug often used to prevent organ transplant rejection. Multiple studies using rodent models have established precedence for rapamycin as an OTC adjuvant, demonstrating that rapamycin protects the PF pool from the effects of increased P13K signaling (97–99). Another study showed that treatment with rapamycin protects PF pools from cyclophosphamide-induced damage (100), suggesting rapamycin may represent a viable pre-OTC treatment adjuvant in patients planning for chemotherapy.

Other pharmacologic treatments targeting mTOR and related PFGA pathways are under investigation. For example, the mTORC1 and mTORC1 and 2 inhibitors everolimus and INK128 have similar positive effects as rapamycin treatments when administered before chemotherapy (101). Other studies have examined the utility of antiapoptotic agents, exogenous antioxidants, and calcium chelators. In a study with cryopreserved human ovarian tissue transplanted in mice, the antiapoptotic agent, carbobenzoxy-Val-Ala-Asp-(O-methyl)-fluoromethylketone, preserved PF numbers, decreased double-stranded DNA breaks, and increased angiogenesis in ovarian tissue grafts (102). Finally, pharmacologic strategies to reduce ROS production have also received attention. Reactive oxygen species are increased during the cryopreservation procedure and are thought to disrupt the homeostatic concentration of Ca^{2+}, perturbing gene expression, protein regulation, and cell growth and death (103–106). Reduction of ROS production has been shown to correspond to enhanced PF survival (107). Both melatonin and the calcium-chelating agent BAPTA acetoxymethyl ester have been shown to reduce ROS when added to cryopreservation solutions (103).

Given the critical role of AMH in the suppression of follicle activation, this physiological PFGA suppressor represents an intriguing adjuvant for OTC/T. Treatment with AMH decreased graft apoptosis in xenotransplanted mice, although an increase in PF reserve was not observed (5, 36, 108). Another study of transplanted human ovarian tissue grafts in mice, cotreated with exogenous endothelial cells and AMH, found improved neovascularization, maintenance of the PF pool, and fewer growing follicles (36, 109). Ultimately, more research is needed to determine whether particular adjuvants deserve a permanent place as fertoprotective additives in standard-of-care OTC procedures.

CONCLUSION

Ovarian tissue cryopreservation is a groundbreaking technology that has made it possible for women who cannot freeze oocytes or embryos before gonadotoxic therapy to preserve their dreams of having a family in the future. However, there remains significant room for improvement in the OTC process. At each point in the OTC procedure, the ovarian reserve is at risk of damage; as such, each step offers potential opportunities for improvement to increase the patient's chance of achieving pregnancy. Through careful selection of freezing methods and transplant locations, as well as consideration of potential adjuvant pharmacologic therapies at multiple stages of the procedure, we can continue to move closer to optimal OTC treatment outcomes for a growing number of patients.

CRediT Authorship Contribution Statement

Abigail Mercier: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data

curation, Conceptualization. **Joshua Johnson:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Amanda N. Kallen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Interests

A.M. has nothing to disclose. J.J. has nothing to disclose. A.N.K. has nothing to disclose.

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