Ovarian tissue transport to expand access to fertility preservation: from animals to clinical practice

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Abstract

Primordial follicles dictate a female's reproductive life span and therefore are central to fertility preservation for both endangered species and individuals with fertility-threatening conditions. Ovarian tissue containing primordial follicles can be cryopreserved and later thawed and transplanted back into individuals to restore both endocrine function and fertility. Importantly, increasing numbers of human live births have been reported following ovarian tissue cryopreservation and transplantation. A current limitation of this technology is patient access to sites that are approved or equipped to process and cryopreserve ovarian tissue – especially in larger countries or low resource settings. Here, we review empirical evidence from both animal models and human studies that suggest that ovarian tissue can be transported at cold temperatures for several hours while still maintaining the integrity and reproductive potential of the primordial follicles within the tissue. In fact, several human live births have been reported in European countries using tissue that was transported at cold temperatures for up to 20h before cryopreservation and transplantation. Ovarian tissue transport, if implemented widely in clinical practice, could therefore expand both patient and provider access to emerging fertility preservation options.

Reproduction (2016) **152** R201–R210

Introduction

Chemotherapy and radiation therapy, although used successfully to treat a myriad of malignant and non-malignant conditions, can have the unintended long-term consequence of damaging reproductive function (Lobo 2005, Meirou *et al.* 2005, Jeruss & Woodruff 2009, Woodruff 2010, Hirshfeld-Cytron *et al.* 2011, De Vos *et al.* 2014, Wallace *et al.* 2014). In females, these treatments can negatively affect several aspects of the reproductive system, including the hypothalamic–pituitary–gonadal axis, the ovary and the uterus (Gracia & Woodruff 2012, Duncan *et al.* 2014). Impaired reproductive function can ultimately result in infertility, sterility and other outcomes, including depression, psychological disorders and sexuality issues (Canada & Schover 2012, Gracia & Woodruff 2012, Lewis *et al.* 2012, Chambers *et al.* 2013, Milbury *et al.* 2013, Lawson *et al.* 2014, Morrow *et al.* 2014, Levine *et al.* 2015). To avoid some of these consequences, fertility preservation strategies have been developed and are being implemented in clinical practice (Waimey *et al.* 2013, De Vos *et al.* 2014).

The American Society for Clinical Oncology (ASCO) and the American Society for Reproductive Medicine

(ASRM), along with the American Academy of Pediatrics and the European Society of Human Reproduction and Embryology (ESHRE), have all recognized the need to address the potential threat of gonadotoxic treatments to fertility, each assembling expert committees and publishing guidance documents emphasizing the need for consultation at the time of diagnosis and rapid referral of patients to reproductive endocrinology and infertility specialists (Ethics Committee of American Society for Reproductive Medicine 2013, Loren *et al.* 2013). To date, female fertility preservation options that are recognized by ASCO and the ASRM/ESHRE are limited to embryo and egg cryopreservation (Ethics Committee of American Society for Reproductive Medicine 2013, Loren *et al.* 2013). Although these assisted reproductive technology (ART) procedures are successful, they may not be available to all patients. For example, embryo and egg freezing are not possible or are contraindicated in prepubertal females or those who cannot delay treatment for their primary condition (Jeruss & Woodruff 2009). For these patients, one experimental fertility preservation option is ovarian tissue cryopreservation (OTC) (Backhus *et al.* 2007, Chen *et al.* 2014, De Vos *et al.* 2014, Dolmans *et al.* 2014, Ross *et al.* 2014,

Stoop *et al.* 2014, Suzuki *et al.* 2015). OTC involves the removal of a whole ovary or cortical biopsies that are then cryopreserved by either slow freezing or vitrification (Rosendahl *et al.* 2011, Ting *et al.* 2013, Suzuki *et al.* 2015). This tissue can be thawed at a later date and used to restore reproductive function (Backhus *et al.* 2007, Chen *et al.* 2014, De Vos *et al.* 2014, Dolmans *et al.* 2014, Ross *et al.* 2014, Stoop *et al.* 2014). OTC followed by orthotopic or heterotopic transplantation has resulted in restoration of endocrine function and fertility, with at least 60 live human births reported to date (Smitz *et al.* 2010, Gamzatova *et al.* 2014, Dittrich *et al.* 2015, Donnez & Dolmans 2015). Although OTC and ovarian tissue transplantation have resulted in live human births, this technology is still considered investigational and may be associated with a reintroduction of the cancer the patient has just survived. As with all experimental technologies, this procedure can only be performed under appropriate ethical protocols.

Despite the investigational designation of OTC, there has been an upward trend in the interest in and use of this technology. In the USA, the National Physicians Cooperative (NPC), which is part of the Oncofertility Consortium, is a nationwide infrastructure of clinical and allied sites committed to providing personalized fertility preservation care (Gracia & Woodruff 2012, Gracia *et al.* 2012) (<http://www.oncofertility.northwestern.edu/health-professionals/npc-membership-info-and-npc-resources>). Several NPC sites offer OTC under institutional review board (IRB)-approved protocols, and the number of OTC cases reported through the NPC has increased steadily in the past decade (Duncan *et al.* 2015). Interestingly, and perhaps not surprisingly, the largest increase in the use of OTC in the NPC has occurred in the pediatric and young adult population, potentially because of increased awareness about this option and the expanding list of medical conditions and treatments that can compromise fertility (Hirshfeld-Cytron *et al.* 2011, Duncan *et al.* 2015).

The ability to perform OTC requires trained staff, specialized equipment, designated space and time to prepare the ovarian cortex and to freeze it using specified protocols (Backhus *et al.* 2007, American Society for Reproductive Medicine 2014). Because of these requirements, the number of clinical sites that offer OTC for fertility preservation remains low. One approach to increasing patient access to OTC is to facilitate efficient ovarian tissue transport. The concept of preserving organ viability during transport has been instrumental for life-saving transplantation of organs, including kidneys, liver, pancreas, lungs and heart. The most common organ preservation technique for transport is induced hypothermia to approximately 4°C (Cantu & Zaas 2011). Hypothermia suppresses metabolism and catabolic enzymes, such that with each 10°C drop in temperature, the metabolic rate is halved (Cantu & Zaas 2011). As a result, at 4°C, the remaining metabolic rate

is approximately 10% of normal (Cantu & Zaas 2011). The lower metabolic rate reduces mitochondrial enzyme activity, which in turn reduces the accumulation of lactic acid and slows down the decrease in intracellular pH, proteolysis, lipolysis and lipid peroxidation associated with ischemia (Guibert *et al.* 2011). Induced hypothermia protects organs from damage while they are removed from the blood supply, but perhaps not surprisingly, the maximum time of preservation at cold temperatures varies depending on the specific organ (Guibert *et al.* 2011). Specifically, the heart (6 h) and the lungs (8 h) have the lowest tolerance for cold ischemia, whereas the liver (12–15 h) and kidneys (24 h) have the highest (Guibert *et al.* 2011).

Ovarian transplantation as a clinical concept has been in existence since the late 1800s; yet, it has lagged behind other organ systems (Rodriguez & Campo-Engelstein 2011). Although cold storage transport is used widely for kidneys, liver, pancreas, lungs and heart, it is not yet standard of care for ovaries in the setting of fertility preservation. However, two European countries – first Denmark and subsequently Germany – have successfully pioneered the clinical use of ovarian tissue transport (Dittrich *et al.* 2012, 2015, Muller *et al.* 2012, Bastings *et al.* 2014, Jensen *et al.* 2015). They describe this model, which has been ongoing for 10 years, as ‘the woman stays – the tissue moves’ (Jensen *et al.* 2015). In this process, ovarian tissue is removed and prepared at a local hospital and then transported to an approved site where the cryopreservation procedure is performed. Following cryopreservation, the tissue is stored for the individual’s future use. Ovarian tissue transport is particularly challenging because there are several structures and cell types within the tissue that need to be maintained in a healthy state. The ovarian cortex is of particular interest because it is enriched in primordial follicles, which comprise a female’s ovarian reserve and dictate her reproductive life span (Schmidt *et al.* 2003a). Once the thawed tissue is transplanted, it is these dormant follicles that will eventually grow, produce hormones and restore reproductive function and fertility. The success of clinical ovarian tissue transport in Europe demonstrates that cold storage of ovarian tissue is efficacious.

Integrating ovarian tissue transport into fertility preservation protocols would expand access to OTC by allowing remote or small clinical practices that may not have resources or staff, the ability to offer this option to their patients. This is especially important in large countries and/or in low resource settings. Additionally, some patients may not have the time to delay treatment for their primary condition in order to travel to a site that offers OTC, or they may not want to seek fertility preservation at a different and/or distant institution. Ovarian tissue transport would overcome these barriers and expand the fertility preservation options available to these patients. In this review, we highlight the

current status of ovarian tissue transport, first discussing research findings in domestic animals and preclinical studies with human tissue, and then presenting clinical results documenting live births following ovarian tissue transport, OTC and transplantation of the thawed tissue. Taken together, there is strong empirical evidence supporting the safe and efficient use of ovarian tissue transport for fertility preservation.

Evidence from animal studies

The mouse is a critical research model, and recently, a comprehensive study was performed that interrogated the effect of storage temperature and duration on ovarian tissue function (Kamoshita *et al.* 2016). Storage at 4°C for up to 24 h did not significantly affect the histological morphology of the tissue or the number of mature gametes that could be collected or fertilized post-orthotopic transplantation (Kamoshita *et al.* 2016). However, cold storage did significantly reduce the incidence of implantation and live offspring (Kamoshita *et al.* 2016). The findings from this mouse study, while important, should be interpreted with caution because the mouse ovary is fundamentally different from ovaries in large mammalian species – especially in terms of architecture and size.

Studies on large animal models have demonstrated that the transport of ovarian tissue is possible without harming the pool of primordial follicles (Baird *et al.* 1999, 2004, Onions *et al.* 2008, Alves *et al.* 2015, Salih *et al.* 2015). Although the majority of studies focus on the health of fully grown oocytes as an end point of ovarian tissue transport (see section on ‘Effect of ovarian tissue transport on oocytes from antral follicles’), some have analyzed the effect of the transport time and temperature specifically on the population of preantral follicles (Silva *et al.* 2000, Lucci *et al.* 2004, Lopes *et al.* 2009, Lima *et al.* 2010, Gomes *et al.* 2012). For example, in goats, transport of ovarian tissue at 4°C for up to 24 h maintained morphologically normal primordial follicles (Silva *et al.* 2000). Once the temperature was increased to 20°C or 39°C, or the time was extended past 24 h, however, there was a significant loss of primordial follicles (Silva *et al.* 2000). These results are consistent with work done on zebu cow ovaries. When ovarian tissue was stored at 4°C for up to 18 h, more than 90% of the preantral follicles exhibited normal structure and morphology (Lucci *et al.* 2004). When the ovarian tissue was stored at 20°C and for longer than 6 h, the morphology of preantral follicles in the ovarian tissue significantly diminished (Lucci *et al.* 2004). In the canine model, one study concluded that ovarian storage at 4°C for up to 12 h provides optimal preservation conditions for preantral follicles and ensures the maintenance of morphology and viability of these follicles (Lopes *et al.* 2009). This observation is supported by another study that found that when

ovarian tissue was held between 3°C and 9°C, more than 80% of the primordial follicles appeared normal and viable. After 36 h at these temperatures, however, only 59.8% of the primordial follicles survived (Lima *et al.* 2010). More recently, a study with equine ovarian tissue concluded that increasing the time and temperature of tissue transport significantly reduced the percentage of morphologically normal follicles (Gomes *et al.* 2012). The greatest percentage of viable follicles was found in equine ovarian tissue that was transported at 4°C for 4 h.

Ongoing work through the NPC is consistent with this published data in large animal models. For example, we have examined the transport of bovine, feline and canine tissue at 4, 25 and 37°C for 24 h. In each of the ovarian tissue types, our histological analysis found that the structure and morphology of primordial and preantral follicles were best maintained when the ovarian tissue was kept at 4 or 25°C (Figs 1 and 2). In our experience, antral follicles were compromised under all transport conditions as evidenced histologically by the disruption of the integrity of the cumulus–oocyte complex (COC) (Fig. 2; see section on ‘Effect of ovarian tissue transport on oocytes from antral follicles’).

Some of the most convincing evidence supporting the clinical use of ovarian tissue transport in the setting of fertility preservation comes from the non-human primate model. A recent study determined that transport of primate ovarian tissue at 4°C for up to 24 h did not compromise the quality of the tissue or the health of the follicles (Hornick *et al.* 2012). When compared with freshly fixed controls, morphology markers in the transported ovarian tissue were indistinguishable and there were no differences in the number of apoptotic cells (Hornick *et al.* 2012). In addition, follicles isolated from these transported tissues survived and grew in an encapsulated *in vitro* follicle growth (IVFG) system that recapitulates oogenesis and folliculogenesis, demonstrating the functionality of these cells after transport (Hornick *et al.* 2012). Thus taken together, there was better preservation of primordial follicles than of developing follicles at colder temperatures in ovarian tissue from most large animal models. Because the majority of primordial follicles are presumably quiescent, it is possible that they may be less affected by the reduced cellular metabolism rate at cooler temperatures (Silva *et al.* 2000, Gomes *et al.* 2012). In addition, the less complex cellular structure as well as the smaller oocyte volume may also contribute to the relative resilience of primordial follicle to cold temperatures.

Evidence from preclinical studies with human ovarian tissue

The ovarian tissue transport studies performed in animal models have been replicated successfully with

Figure 1 Ovarian tissue transport at colder temperatures maintains primordial follicle morphology in multiple species. Bovine (A–D), canine (E–H) and feline (I–L) ovarian tissue was harvested from individual animals and divided. One piece of tissue was fixed immediately upon organ removal (A, E and I), and the remaining pieces were transported at 4°C (B, F and J), 25°C (C, G, K) and 37°C (D, H and L) for 24 h. In this manner, the only variable was transport temperature. Following transport, the ovarian tissue was fixed and the morphology of the primordial follicles within the ovarian cortex was examined by standard histological evaluation by hematoxylin and eosin staining. Primordial follicle integrity was compromised in all species following transport at 37°C. However, primordial follicles at both 4 and 25°C looked similar to fresh fixed controls. These experiments were repeated a minimum of three times and representative images are shown. Scale bar = 50 µm. The insets are higher magnification images, which highlight the morphology of the primordial follicles.

human ovarian tissue by several groups (Schmidt *et al.* 2003b, Isachenko *et al.* 2009, Laronda *et al.* 2014). The transport of human ovarian tissue at cold temperatures and within a period of 4–26 h maintains functional parameters of the tissue as assessed by morphology, viability and follicle development *in vitro* and *in vivo* (Schmidt *et al.* 2003b, Isachenko *et al.* 2009, Laronda *et al.* 2014). In one recent study, ovarian cortical tissue from 24 individuals was transported for up to 22 h at 4°C, and cell quality and morphology were compared in histological and TUNEL analyses to pre-transport control tissue from the same individual (Laronda *et al.* 2014). In more than half of the samples, the transported tissue was histologically similar to the freshly fixed control tissue, and TUNEL analysis demonstrated a similar percentage of apoptotic cells in the pre- and post-transport tissues. In some samples, significantly more TUNEL-positive cells were observed in the post-transport tissue compared with freshly fixed control tissue. These TUNEL-positive cells, however, were primarily constrained to the edge of the tissue. Preantral follicles at the secondary follicle stage isolated from this transported human ovarian tissue were able to survive and grow to the antral stage

in culture and to develop with normal morphology (Laronda *et al.* 2014).

The findings from the study by Laronda *et al.* (2014) are consistent with another study that also evaluated the quality of transported human ovarian tissue using an IVFG assay (Isachenko *et al.* 2009). In that study, storage of ovarian cortical fragments at suprazero temperatures for up to 26 h did not compromise the *in vitro* development of follicles during subsequent culture of intact tissue pieces (Isachenko *et al.* 2009). Interestingly, the storage of human ovarian cortex at 5°C for 24 h before cryopreservation increased the viability of the follicles after thawing as assessed by culture in the chorioallantoic membrane system followed by immunohistochemical analysis of proliferation marker expression within the cultured tissue (Isachenko *et al.* 2013). Immunohistochemical analysis of ovarian tissue after 24 h at 5°C also showed that neovascularization was increased compared with non-cooled control tissue, which is important because this process is critical for implantation and function of transplanted ovarian tissue (Isachenko *et al.* 2012). The physiological basis for these phenomena, however, is unclear. Moreover, without evaluation of the number and viability of follicles as

Figure 2 Ovarian tissue transport at colder temperatures maintains preantral follicle morphology in multiple species. Bovine (A–D), canine (E–H) and feline (I–L) ovarian tissue was harvested from individual animals and divided. One piece of tissue was fixed immediately upon organ removal (A, E and I), and the remaining pieces were transported at 4°C (B, F and J), 25°C (C, G and K) and 37°C (D, H and L) for 24 h. In this manner, the only variable was transport temperature. Following transport, the ovarian tissue was fixed and the morphology of the preantral follicles within the ovarian tissue was examined by standard histological evaluation by hematoxylin and eosin staining. Preantral follicle integrity was significantly compromised in all species following transport at 37°C. However, preantral follicles at both 4 and 25°C looked similar to fresh fixed controls. Antral follicles did not appear to tolerate any transport conditions. This is highlighted in the feline sample where disruption of the cumulus layer surrounding the oocyte was evident following transport (J and K) but not in the control (I). These experiments were repeated a minimum of three times and representative images are shown. Scale bar = 50 µm.

well as the extent of vascularization in the tissue pieces before experimental manipulation, it is impossible to conclude whether cooling *per se* had a significant effect on these parameters.

Reports of consistent histology and IVFG following transport are robustly supported by the successful xenotransplantation of human ovarian tissue that was transported before cryopreservation (Schmidt *et al.* 2003b). Following transport on ice for approximately 4 h, ovarian tissue was cryopreserved, then thawed and xenotransplanted into a mouse. After 4 weeks, each of the transplanted pieces of ovarian tissue was recovered and found to contain healthy follicles at the primordial, primary and secondary stages (Schmidt *et al.* 2003b). This work is of particular significance because it demonstrates the function of the transported, cryopreserved, thawed and transplanted tissue in an *in vivo* model. Taken together, this research demonstrates that transport of human ovarian tissue at or around 4°C before cryopreservation maintains the primordial follicle pool and may even have a positive effect on the subsequent growth and development of the follicles within the tissue (Schmidt *et al.* 2003b, Isachenko *et al.* 2013, Laronda *et al.* 2014).

Clinical studies

The strongest unequivocal support of the safety and efficacy of ovarian tissue transport are the live births that have been reported after transplantation of transported ovarian tissue. Transplantation of ovarian tissue has led to at least 60 live births to date, and several of these were achieved after transplantation of ovarian tissue that was transported for up to 22 h before cryopreservation (Andersen *et al.* 2008, Ernst *et al.* 2010, Rosendahl *et al.* 2011, Dittrich *et al.* 2012, Muller *et al.* 2012, Dittrich *et al.* 2015, Donnez & Dolmans 2015, Jensen *et al.* 2015). These cases indicate that transport before cryopreservation does not compromise the functional potential of the tissue. Moreover, these reports demonstrate that (1) live births can be obtained following cold tissue transport at 4°C, (2) tissue can be transported up to 20 h and remain viable and (3) multiple live births can be obtained from the same tissue.

In several European countries, primarily Denmark and Germany, ovarian tissue transport has been combined with OTC and transplantation with successful clinical outcomes. In Denmark, OTC is only performed at the Laboratory of Reproductive Biology at Copenhagen

University Hospital, Rigshospitalet, which provides a centralized localization (Rosendahl *et al.* 2011). Ovaries removed from patients at other centers around the country must be placed on ice and transported by airfreight, train or car within 4–5 h to the Copenhagen University Hospital for cryopreservation. As of 2015, this program performed 53 transplantations to 41 patients over the course of 10 years, and nine of these women had a tissue that was transported on ice before cryopreservation (Jensen *et al.* 2015). This group reports a pregnancy rate of ~30% using this technology, again supporting the combined use of ovarian tissue transport with OTC and transplantation (Jensen *et al.* 2015).

Two case reports from the Danish and German experiences are described in more detail below because they highlight the longevity and functionality of ovarian tissue grafts post-transport and illustrate the relative resistance of ovarian tissue to cold transport. In the first case from Denmark, a 27-year-old woman who had been diagnosed with Ewing's sarcoma had undergone OTC for fertility preservation. The patient's ovarian tissue was transported on ice for 5 h and then cryopreserved for 21 months. For the ovarian transplantation procedure, tissue corresponding to approximately 15–20% of one entire ovary was transplanted into subperitoneal pockets in the patient's parietal peritoneum (Andersen *et al.* 2008, Ernst *et al.* 2010). This woman had two healthy girls from this tissue, making her the first patient to have two separate pregnancies and births resulting from a single OTC–autotransplantation procedure. At the time of OTC, the woman only had her right ovary because the left one had been removed previously due to a dermoid cyst. A part of the right ovary was removed for OTC. Although it is possible that the pregnancies resulted from residual ovarian tissue rather than the transplanted tissue, a biopsy of the remaining ovary was performed at the time of transplantation, and no follicles were found by histological examination.

In the second case from Germany, a live birth following ovarian tissue cryopreservation and transplantation was reported after 20 h of cold transport, suggesting that the ovary is similar to the kidneys and liver in terms of its tolerance to cold temperatures (Dittrich *et al.* 2012, Guibert *et al.* 2011). In this case, a 25-year-old woman with nodular sclerosing Hodgkin lymphoma had two-thirds of the ovarian cortex from each ovary removed, and the tissue was transported at 5–8°C for 20 h from Dresden University Hospital to the Reproductive Medicine Laboratory in Bonn for cryopreservation (Dittrich *et al.* 2012). After 5 years of cryopreservation, the tissue was transplanted into a deep pouch of the peritoneum in the region of the broad ligament. The patient had a spontaneous pregnancy and delivered a healthy male (Dittrich *et al.* 2012). During the cesarean section, the research team stated that healthy follicles were clearly visible on the surface of the transplanted tissue (Muller *et al.* 2012). Further, biopsy samples

taken from the transplanted ovarian tissue revealed numerous follicles in all stages of development. This histological analysis confirms the viability of ovarian tissue that had been transported up to 20 h at 4°C before cryopreservation (Muller *et al.* 2012).

Thus, it is clear that in the short time that ovarian tissue transport protocols have been employed in Europe, there have been multiple live births following autotransplantation (Andersen *et al.* 2008, Ernst *et al.* 2010, Rosendahl *et al.* 2011, Dittrich *et al.* 2012, Muller *et al.* 2012, Dittrich *et al.* 2015, Jensen *et al.* 2015). These impressive clinical results illustrate the potential for integrating ovarian tissue transport before cryopreservation as a way to expand global access to OTC.

Effect of ovarian tissue transport on oocytes from antral follicles

The majority of this review has focused on ovarian tissue transport conditions that favor the preservation of preantral follicles. However, ovarian tissue also contains antral follicles at various stages of development. Cumulus–oocyte complexes (COCs) from both small and large antral follicles can be isolated directly from harvested ovarian tissue and matured *in vitro* to obtain mature gametes. In fact, COCs in the ovarian medulla are often released from antral follicles during cortical ovarian tissue processing for OTC and can be used for fertility preservation if they are from follicles that have reached adequate maturity and if they are of high quality (Revel *et al.* 2003, Duncan *et al.* 2012). Mature eggs derived from IVM can be cryopreserved or fertilized and then cryopreserved as embryos. *In vitro* maturation (IVM) is another promising fertility preservation option, and importantly, pregnancies and live births have been obtained using this method (Guzman *et al.* 2012, Chian *et al.* 2013, Segers *et al.* 2015, Uzelac *et al.* 2015).

In animal studies, the data regarding how well COCs withstand cold transport are not consistent. In one study, bovine ovaries were transported for 3–4 h at 15, 25 and 35°C; oocytes from the ovaries transported at 15°C had a higher developmental competence and lower apoptotic index compared with the other transport groups (Wang *et al.* 2010). In the canine model, oocytes obtained from ovaries transported for up to 4 h at 4°C had a significantly higher MII maturation rate than those transported at 35–38°C (Tas *et al.* 2006). However, in another study, transport at 37°C for up to 8 h resulted in the greatest oocyte viability, although this study did not directly compare outcomes with oocytes from ovarian tissue transported at 4°C (Hanna *et al.* 2008). Conflicting results regarding COC viability after ovarian tissue transport have been seen in other animal models as well. One study concluded that when equine ovaries were transported within 7 h, temperature played no role in determining the developmental competence of retrieved

oocytes (Ribeiro *et al.* 2008), whereas another group reported that transport at 4°C for up to 4 h was optimal for oocyte viability (Gomes *et al.* 2012). Experimental data from the feline model showed that oocytes from ovaries transported for up to 24 h at 4°C were capable of producing blastocysts after *in vitro* fertilization (Wolfe & Wildt 1996, Naoi *et al.* 2007, Evecen *et al.* 2009). The effect of cooler transport temperatures was also seen in a study with Iberian red deer, which showed that ovarian transport for up to 12 h at 5–8°C produced oocytes that had a higher embryonic cleavage rate than those transported at 20–25°C (Garcia-Alvarez *et al.* 2011).

In humans, the data be more conclusive that COCs do not withstand extended transport at cold temperatures well. Duncan *et al.* (2012) reported that although COCs can be isolated directly from fresh human ovarian tissue, there were no viable COCs recovered from tissue that had been transported for 22 h at 4°C. Short-term transport before OTC has also been shown to significantly compromise the oocyte maturation rate (Wilken-Jensen *et al.* 2014). Cryopreserved ovarian tissue from a cohort of 61 patients (0–38 years of age) from Denmark's centralized cryopreservation program was examined; the tissue transport time for these samples was between 2 and 5 h at 4°C. A total of 682 immature oocytes within cumulus–oocyte complexes (COCs) were collected; 11% of oocytes from non-transported

tissue were successfully matured *in vitro* to the MII stage compared with 1.4 and 4.6% of oocytes from tissue that had been transported 2–3 h and 4–5 h respectively.

Taken together, these results suggest that there are species-specific and follicle class-dependent differences in optimal ovarian tissue transport conditions. For human ovarian tissue, cold temperatures favor the preservation of preantral follicles but may not be optimal for antral follicles.

Summary and future directions

In summary, critical advances have been made in ovarian tissue transport. Preclinical studies support the use of this technique in the setting of fertility preservation for both endangered species and patients with fertility-threatening conditions (Fig. 3). Studies are ongoing to further optimize ovarian tissue transport conditions. For example, in studies using sheep ovaries as a model, addition of anti-apoptotic agents in the transport, processing and cryopreservation media improved the quality of primordial follicles post-thaw as assessed by culture and histological evaluation (Henry *et al.* 2016). It is especially encouraging for the field of fertility preservation that multiple human live births have been reported following transplantation of transported ovarian tissue. This technique, should it be

Figure 3 Schematic detailing how ovarian tissue transport integrates into clinical fertility preservation. Ovarian tissue transport would expand the fertility preservation options to those individuals who currently do not have access. Ovarian tissue that is removed as an entire organ or as a biopsy would be prepared into cortical strips upon harvesting and transported to an approved processing site. Upon arrival at the processing site, the ovarian tissue could be cryopreserved by slow freezing or vitrification methods. Following thawing, the ovarian tissue could be used for transplantation to restore endocrine function and/or fertility. Ovarian follicles could also be isolated from the thawed ovarian tissue and used for *in vitro* follicle growth (IVFG) to obtain mature eggs that can be used to generate embryos following *in vitro* fertilization or intracytoplasmic sperm injection. Another option is that follicles could be isolated directly from the transported tissue without the need for tissue cryopreservation. These isolated follicles could be cryopreserved or used for IVFG.

implemented in clinical practice in larger countries or in those with limited resources, will increase the options for patients who are in geographic areas that lack an oncofertility program to have ovarian tissue preserved for their later use for transplantation or other emerging fertility preservation options (Fig 3).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work was funded through the Thomas J Watkins Endowment (TKW, Northwestern University, Chicago, IL, USA) and by the Center for Reproductive Health After Disease (P50HD076188) from the National Institutes of Health National Center for Translational Research in Reproduction and Infertility (NCTRI).

Acknowledgements

The authors would like to thank Dominique Keller for useful insight into ovarian tissue transport studies in domestic species. They also acknowledge the Aurora Packing Company, Jessica Hornick, and Jennifer Nagashima for technical assistance with the bovine, canine and feline experiments. They appreciate the work of the National Physicians Cooperative and particularly Kristin Smith and Brigid Martz. Finally, they would like to thank Stacey Tobin for editorial assistance and Stanton Fernald for his expertise in medical illustration.

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Received 17 December 2015

First decision 2 February 2016

Revised manuscript received 28 June 2016

Accepted 4 August 2016