

DEBATE—continued

Ovarian tissue banking for cancer patients

Reduction of post-transplantation ischaemic injury: intact ovary freezing and transplantation

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Despite reasonable achievements in different animal species, the debate about many technical aspects of ovarian tissue banking is continuing. Human ovarian tissue banks are increasingly established around the world without a clear plan about how to make the best use of such tissue. One of the important challenges facing this growing technology is to determine the ideal method for the use of this cryopreserved ovarian tissue. It is not uncommon in medicine to introduce a technology without a clear understanding of the consequences. If it is decided that ovarian tissue is to be autotransplanted, what is the most suitable place? Which technique should be implemented? As a part of the ongoing debate on ovarian tissue banking in cancer patients, this paper supports the notion that cryopreservation of an intact ovary with its vascular pedicle may be a viable alternative to the currently available techniques. Research in the development of technology to cryopreserve whole organs as well surgical techniques for the autotransplantation of an ovary with its vascular pedicle should be encouraged.

Key words: cancer/fertility preservation/ischaemic injury/ovarian freezing/ovarian tissue banking

Introduction

Ovarian cryopreservation and transplantation is a much-debated experimental procedure (Kim, 2003; Revel and Schenker, 2004; Oktay and Sonmeyer, 2004) introduced to preserve fertility in women with threatened reproductive potential (Oktay and Buyuk, 2002). Gosden and associates demonstrated that the sheep provides a useful model to study ovarian tissue cryopreservation and transplantation. Using cryopreserved–thawed ovarian cortical strips, they showed follicular survival and endocrine function, as well as restoration of fertility after transplantation of cryopreserved–thawed ovarian cortical strips (Gosden *et al.*, 1994; Baird *et al.*, 1999). The success achieved in orthotopic transplantation of cryopreserved ovarian cortical strips from both mouse and sheep resulted in the subsequent experimental use of this technique in human reproductive medicine.

Using present techniques, ischaemic injury to the transplanted tissue results in the loss of virtually the entire growing follicle population and a significant number of primordial follicles (Newton *et al.*, 1996). This could limit its long-term viability. Alternatively the primordial follicles in the ovarian tissue can be matured *in vitro* or in an immune-deficient animal host. The former cannot be accomplished with present technology and the latter has significant ethical and biological issues, such as transmission of virus or viral particles. The focus of this contribution will be about the potential of

cryopreserving an intact ovary followed by its transplantation. Although the published work is encouraging, many technical and laboratory nuances need to be resolved.

Which cryoprotective agent to be used?

The early work by Newton *et al.* (1996) demonstrated that human ovarian tissue lost 90% of the primordial follicle population on using glycerol as a cryoprotective agent (CPA) compared to 25, 15 and 55% on using dimethylsulphoxide (DMSO), ethylene glycol (EG), and polyethyleneglycol (PG) after transplantation in severe combined immunodeficient (SCID) mice respectively. This was the first xenografting study, grafting 1–2 mm³ ovarian cortical strips in SCID mice (Newton *et al.*, 1996). Such hazardous effect of glycerol was explained by the low permeability coefficient of glycerol compared to DMSO and EG or PG. Currently, DMSO is one of the most frequently used CPA for ovarian cryopreservation (Gosden *et al.*, 1994; Baird *et al.*, 1999).

Similar results were obtained by Candy *et al.* (1997). They suggested that to achieve optimal cryoprotection, it is essential that freezing protocols allow uniform penetration of CPA throughout the ovarian tissue. Consequently, the CPA permeation rate is an important limiting factor in developing better cryopreservation protocols for ovarian tissues (Candy *et al.*, 1997). Besides the importance of the diffusion rate, Newton *et al.* (1998) also studied the importance of the diffusion

temperature. They concluded that at a higher temperature (37°C) all CPA penetrate the ovarian tissue at a faster rate. Recent evidence shows that using such a protocol will result in the development of a metaphase II oocyte from xenografting cryopreserved–thawed human ovarian cortical strips in immunodeficient mice (Gook *et al.*, 2003).

Tissue penetration of cryoprotectant in bigger pieces of tissue or whole organ systems is even more complicated. The transplant literature, such as with kidneys, have several protocols that strive to protect the tissue prior to transplantation. These protocols try to perfuse the whole organ with a CPA. The potential for cryopreserving the entire ovary has the same methodological limitations. The cryoprotectant can be perfused through the ovarian vessels using a special pump to achieve the appropriate tissue penetration.

The fact that the ovary has different heterogeneous cellular components adds to the technical challenge of intact ovary freezing as different cell types have different properties which may alter their requirements for cryoprotectants. Consequently, the differential effect of a single CPA on the epithelial, germ and sex cord–stromal cell components of the ovary may affect their post-thaw survival and function. However, unlike the disappointing results of earlier trials of kidney cryopreservation, using this approach, pregnancies in rats (Wang *et al.*, 2002) and partial restoration of hormonal function in sheep were recently reported (Bedaiwy *et al.*, 2003).

Vascularized ovarian grafts: is it possible?

The major limiting factor for the survival of primordial follicles in the transplanted graft is the revascularization time. In an attempt to minimize follicular loss and maintain the function of the ovarian grafts, transplantation of an intact ovary with microvascular anastomosis has been attempted in an animal model. This will maximize the possibility of immediate vascularization and potentially minimize post-transplantation ischaemic follicular demise. Although whole ovaries from mice and rats survive freezing because of their smaller sizes, successful cryopreservation of whole ovaries from other mammalian species such as human and non-human primates is technically more challenging. Typically, the human ovary is 4×2×0.8 cm, 20–35 g, compared to the sheep ovary which is 2.5×1.5×0.5 cm, 3–8 g. This substantial difference in volume may influence the penetrance of the cryoprotectant.

In our trials to establish an animal model for studying different aspects of vascularized ovarian grafts, we proposed anastomosis of the ovarian vessels to the deep inferior epigastric vessels in a sheep model. The inferior epigastric vessels are branches of the external iliac artery that lie superior to the inguinal ligament and posterior to the rectus muscle. They are easily accessible without entering the peritoneal cavity. Furthermore there is an extensive experience in the plastic surgery literature in the use of these vessels for breast reconstructive surgery. Normal hormonal functions, higher primordial follicular counts as well as normal ovarian histological architecture were observed in animals with patent vascular anastomosis compared to those with occluded anastomosis. This experiment proved the feasibility of using

the deep inferior epigastric vessels as a potential recipient site for vascularized fresh ovarian grafts (Jeremias *et al.*, 2002). It provides an easy access to the microvascular anastomosis technique as well as future oocyte harvesting.

Similarly, in another experiment we tested the feasibility of transplanting intact frozen–thawed ovary with microvascular anastomosis of the ovarian vascular pedicle to the deep inferior epigastric vessels using the same technique. No significant differences were observed between the mean values of apoptosis and follicular viability in cryopreserved–thawed ovarian cortical strips placed along the course of the deep inferior epigastric vessels without vascular anastomosis and cryopreserved–thawed intact ovaries, demonstrating that cryosurvival of whole ovary is as good as cortical strips, at least in the sheep. After autotransplantation of the whole organ, post-operative FSH and serum estradiol levels were similar to pre-operative values within 1 week in animals with patent vascular anastomosis at necropsy. From this study we concluded that transplantation of intact frozen–thawed ovary is technically feasible. Using this approach, immediate restoration of vascular supply and ovarian hormonal function is possible (Bedaiwy *et al.*, 2003). These provisional results should also be documented in long-term studies in humans. Although there is some question of systemic thrombotic risks after vascular transplantation, this has not been the experience in the reconstructive plastic surgery literature, where extremely complex autografting with a vascular anastomosis is used routinely in plastic surgery, with few life-threatening complications. Moreover, pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation was reported (Racho El-Akouri *et al.*, 2003).

Autografting of human ovarian tissue: current status and limitations

Several groups (Oktay and Karlikaya, 2000; Callejo *et al.*, 2001; Oktay *et al.*, 2001; Radford *et al.*, 2001) have attempted autografting human ovarian cortical strips into orthotopic or heterotopic locations with some short-term function but with no documented fertility resumption. Oktay *et al.* (2004) have reported the development of one 4-cell embryo after heterotopic transplantation of cryopreserved ovarian tissue. After eight unstimulated and stimulated cycles, 20 oocytes were retrieved and eight were suitable for IVF. No pregnancy occurred after transfer of the embryo. Although unsuccessful in the human so far, a recent report of a live birth in a monkey model using the technique of autografting ovarian tissue pieces without a vascular supply to the abdominal wall has recently been reported (Lee *et al.*, 2003).

The potential for whole organ transplant

Given the above-mentioned limitations of fresh and cryopreserved–thawed ovarian cortical strips, intact ovarian tissue grafting in conjunction with cryopreservation is thought to have the potential to preserve fertility in patients at risk of premature ovarian failure. Until now, autotransplantation procedures are almost exclusively limited to non-vascular

cortex segments grafted to either orthotopic or heterotopic locations. Non-vascular grafts sustain significant ischaemic injury until revascularization of the tissue takes place. Reduction of the ischaemic time-interval after reimplantation is crucial for the reproductive potential of these transplants. A vascular transplant is the logical approach. Despite the technical difficulty of the transplantation procedure, it grants immediate blood supply to the transplant, thus minimizing ischaemic injury. We believe that immediate revascularization of ovarian transplants can be achieved by performing a vascular anastomosis. The time-period from the laparoscopic oophorectomy until completion of the autotransplantation procedure (warm ischaemia time) in our experiments was ~4 h.

Another limitation for intact organ transplantation is the need for the removal and storage of an intact ovary or an ovarian segment with its vasculature. Several technology companies are working on developing cryo-chambers that will hold the size of a human ovary. Cryopreservation protocols are currently almost exclusively limited to fragmented non-vascular cortex pieces. However, in recent years improvements in freezing protocols for vessel grafts, and reports of the cryopreservation of composite tissue and animal organs, point to the possibility of organ cryopreservation. Our preliminary data support this observation (Bedaiwy *et al.*, 2003).

Conclusions

It is critical for patients to understand that no pregnancies have been reported in humans with these techniques. Research should focus on refinement of the cryopreservation protocols, better cryoprotectants and transplantation techniques that decrease ischaemia. Selection of the transplantation site should consider easy, simple and minimally invasive surgical technique. Moreover, ample blood supply to the recipient site is important for graft establishment, survival and long-term function. Subsequent manipulations of the grafted ovary, as in follicle aspiration, should be made simple.

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