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COMMENTARY

Time to re-think: ovarian tissue transplantation versus whole ovary transplantation


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Abstract Transplantation of cryobanked ovarian tissue is a promising strategy to restore fertility in cancer patients. However, ischaemia following ovarian tissue grafting can lead to significant follicular loss. Transplantation of the whole ovary by vascular anastomosis has been considered as a method of preventing ischaemic damage that occurs with avascular transplantation of ovarian tissue. Even so, the unavailability of the cryotechnology for whole organs can be a major barrier to whole ovary transplantation. Severe cryoinjury will cause not only follicular death but also irreversible damage to the vascular system of the ovary. Damaged ovarian vasculatures can induce thromboembolism after transplantation which leads to severe tissue ischaemia and follicular loss. As a consequence, follicular loss after the frozen–thawed whole ovary was transplanted with microsurgical vascular anastomosis has been shown to be as severe as that which occurred after ovarian tissue was grafted. In addition, the risk of cancer cell reintroduction can be potentially higher with whole ovary transplantation with vascular anastomosis. The safety and efficacy of the new procedure should be proven before any further clinical applications take place. Nevertheless, research on whole ovary cryopreservation should not be discouraged. 

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Introduction

Advances in cancer therapy have resulted in a steady increase in the long-term survival. Of course, fertility preservation is perceived as a crucial quality of life issue in young cancer survivors. One of the emerging strategies to preserve fertility is ovarian cryopreservation and transplantation. A

current increase in enthusiasm and awareness of this technology is encouraging, but a critical analysis based on evidence will be required to develop successful clinical and research programmes for ovarian cryopreservation and transplantation.

Although still in its early stages, ovarian tissue cryopreservation followed by transplantation has proven to be

successful in several instances. To date, six healthy babies have been born worldwide after grafting frozen–thawed ovarian tissue in cancer patients (Andersen et al., 2008; Demeestere et al., 2007; Donnez et al., 2004; Meirou et al., 2005; Piver et al., 2009). The main problem with ovarian tissue transplantation is ischaemic tissue damage while waiting for angiogenesis to the graft. To date, we do not have good strategies to minimize ischaemic damage. Facilitating angiogenesis by genetic manipulation of angiogenic factors, alleviation of hypoxic damage with antioxidants (Kim et al., 2004) or transplantation by vascular anastomosis can be potential solutions in the future.

In theory, ovarian transplantation by vascular anastomosis can minimize ischaemic injury. However, significant ischaemia–reperfusion injury to the transplanted ovary occurs even with vascular reanastomosis. The greatest risk to follicular survival is thromboembolism after vascular transplantation. In addition, imperfect freezing technologies for bulky organs can be a big barrier of whole ovary transplantation. Although it is exciting that a healthy baby girl was born after microsurgical transplantation of an intact ovary between monozygotic twins (Silber et al., 2008), the benefits and risks of this procedure should be thoroughly evaluated at this point, since implementation of this new technology without clear evidence of benefits may prove to be harmful and unethical.

Historical background

The history of ovarian transplantation dates to the 18th century. Although many animal experiments were performed in 19th century Europe, the first human ovarian tissue transplantation was reported by Robert Morris in New York in 1895 (Morris, 1895). Transplantation of the whole ovary with vascular anastomosis is not a new procedure either. In 1906, Alexis Carrel in New York, who later won a Nobel Prize, reported the first ovarian transplantation by vascular anastomosis in cats. Since then, successful transplantation of the whole ovary with microanastomosis of vascular pedicles has been reported in many animals, including dogs, cats, rodents, rabbits, sheep and primates. In 1987, Michel Leporrier in France (Leporrier et al., 1987) reported the successful heterotopic transplantation of the whole ovary with vascular anastomosis before pelvic irradiation to treat Hodgkin's disease, the first successful whole ovary transplantation in humans, demonstrating the feasibility of its clinical application.

Can vascular transplants improve follicular survival?

Transplantation of ovarian tissue is less technically challenging than transplantation of the whole ovary by microsurgical anastomosis of vascular pedicles. Autotransplantation of frozen–thawed human ovarian tissue has been proven to restore endocrine function as well as fertility. Nevertheless, problems remain with this technology, such as ischaemic injury and the possibility of cancer cell reintroduction. The most urgent issue to address before full clinical application of this technology is prevention of ischaemia

to improve follicular survival in the ovarian graft. It has been known that follicular loss after transplantation of ovarian tissue is over 50%. Therefore, many researchers have been investigating different strategies to minimize ischaemic injury in the ovarian graft, such as applying antioxidants and angiogenic factors (Kim, 2006).

In theory, whole intact ovary transplantation with vascular anastomosis appears to be a logical solution for ischaemic damage. The most data, however, do not support this theory. Courbiere et al. (2009) reported successful microsurgical transplantation of the fresh intact ovary in ewe, but follicular survival rate was only 6%. This poor follicular survival is most likely due to prolonged warm ischaemia and significant thrombosis.

Although ovarian vascular pedicles in humans are larger and less tortuous compared with sheep ovarian vessels, vascular transplantation of the whole human ovary is still technically challenging (especially end-to-end anastomosis). Of note, the size of human ovarian arteries is between 0.5 mm and 1.0 mm in diameter. In fact, the reported ovarian warm ischaemic period during vascular transplantation was over 100 min in both ewes and humans (Courbiere et al., 2009; Silber et al., 2008). Nevertheless, restoration of ovarian function (Mhatre and Mhatre, 2006) and successful pregnancy (Silber et al., 2008) after orthotopic transplantation of the fresh whole ovary by vascular anastomosis have been reported in humans.

The results of transplantation of the frozen–thawed whole ovary are even more disappointing. Microvascular anastomosis of the whole cryopreserved ovary in sheep revealed large fibrotic areas with absence of follicles (30–50%) in the transplanted ovary. As a consequence, the follicular survival rate was less than 8% (Imhof et al., 2006). This severe follicular loss can be caused not only by direct cryoinjury to the follicles due to suboptimal cryotechnology, but also by ischaemia induced by thromboembolism in the vascular system of the ovary after transplantation.

Suboptimal cryopreservation of the whole organ will cause significant vascular damage that can lead to micro- and macrothromboembolism after transplantation. Viability data following whole ovine ovary cryopreservation have shown a significant detrimental effect of cryopreservation on the extent of arterial endothelial cell layer detachment and arterial smooth muscle damage (Onions et al., 2008). In fact, Bedaiwy et al. (2003) demonstrated poor long-term vascular patency after autotransplantation of intact frozen–thawed ovine ovaries with microvascular anastomosis. The anastomosed vessels were completely occluded in eight of 11 cases, leading to immense follicular loss.

Why is it so difficult to freeze the whole organ?

With advances in transplantation medicine, organ transplantation has become a common surgical procedure. As such, the complexity of surgical technique is no longer a barrier to transplanting the whole ovary with vascular anastomosis. The major obstacle for the whole ovary transplantation remains the inadequate freezing technique for the whole organ, as freezing and thawing can destroy cellular ultrastructures and subsequently their functions. In particular, vascular injury with freezing and thawing can be of concern.

When multicellular systems are freezing, extracellular ice can be as lethal as intracellular ice. In fact, there has been no documented success in freezing vital organs (such as livers and kidneys) because of the difficulty of preventing extracellular ice formation, particularly the formation of intravascular ice.

Additional factors also make cryopreservation of the organ difficult. As the organ is composed of many different cell types and bulky in volume, delivering adequate concentrations of cryoprotectant into all of cells in a timely manner is a formidable challenge.

Vascular perfusion has been used to deliver cryoprotectants evenly to all cells in the organ, although its efficacy is still questionable. Our study showed that cryoprotectant penetration to the ovary (assessed by nuclear magnetic resonance spectroscopy) did not improve after 30-min perfusion in rat (Yin et al., 2003). Vascular perfusion can be destructive to the capillary endothelial cells because of chemical and osmotic toxicities. Also, changes in cell volume during perfusion can increase vascular resistance in ways that compromise the perfusion process itself. It would be advantageous to select a cryoprotectant with high permeability and to minimize its concentration. In addition, keeping the perfusion pressure below 30 mmHg is crucial to minimizing the mechanical damage to the capillaries (Lachenbruch et al., 1998).

Another barrier of organ cryopreservation is the restriction of cooling and heating rates to those that are permitted by the dimensions and geometry of the organ. Indeed, all different types of cells in a proper three-dimensional arrangement should be well preserved to restore normal organ function, since the function of the organ depends on interrelations between the cells. The rate at which heat penetrates the bulky organ is dependent on its thermal conductivity and heat capacity (Pegg, 2005). It is a real challenge to control the heat conductivity to achieve the even distribution of heating and cooling energy to the whole organ.

What are advantages and disadvantages of the vascular transplantation of the frozen–thawed whole ovary?

The major difficulty with ovarian tissue transplantation is significant follicular loss as a consequence of ischaemia

while waiting for neovascularization to the ovarian graft. Transplantation by vascular anastomosis, on the other hand, can restore blood supply immediately after transplantation. By minimizing ischaemia time with whole ovary transplantation, greater longevity of ovarian function can be expected. Unfortunately, follicular loss after vascular transplantation of the whole ovary is as severe as after avascular transplantation of ovarian tissue because of the microvascular injury and thrombosis leading to cell death.

Even with ischaemia, frozen–thawed ovarian tissue transplanted without vascular anastomosis can maintain long-term survival. Kim et al. (2009) reported restoration of long-term ovarian function lasting more than 4 years after transplantation of frozen–thawed human ovarian tissue, which is longer than that of frozen–thawed whole ovary transplants reported in the literature. Furthermore, one whole ovary can be processed to numerous sections of ovarian cortex and cryopreserved in separate ampoules, which provides the option of repeated transplantation to prolong ovarian function and fertility in cases where the first transplantation fails or ceases to function. This flexibility is lacking with vascular transplantation of the whole ovary that is subject to an all-or-nothing principle. Thus, currently no evidence has been documented of advantages of transplanting the whole frozen–thawed ovary by vascular transplantation.

On the contrary, many disadvantages of vascular transplantation of the frozen–thawed whole ovary are evident (Table 1). First, successful cryopreservation of the whole organ has not been achieved and it will take time to perfect this technology. Second, ischaemia–reperfusion injury cannot be avoided even with vascular transplantation. Indeed, surgical time can be prolonged due to the technical complexities of vascular surgery, which can accelerate follicular loss through warm ischaemia. Moreover, thromboembolism after vascular transplantation can cause extensive cell death and be life threatening under certain circumstances. In many animal studies, increased mortality and morbidity after whole ovary transplantation have been observed (Grazul-Bilska et al., 2008; Yin et al., 2003). Finally and importantly, whole ovary transplantation can increase cancer cell reintroduction in cases of high risk of residual ovarian medullary pathology to a greater degree than can transplantation of small pieces of ovarian cortical sections. Although the safety of grafting ovarian cortical tissue from lymphoma patients has been demonstrated (Kim et al., 2001), it is

Table 1 Advantages and disadvantages of whole ovary transplantation.

Advantage	Disadvantage
Immediate blood supply to the graft	Difficulties of organ cryopreservation
Minimizing tissue ischaemia	Surgical complexities and longer surgical time
Possible long-term ovarian function	Ischaemia–reperfusion injury Thromboembolism Increased morbidity and mortality Risk of reintroduction of cancer cells (especially with minimal residual disease in ovarian medulla)

unknown if ovarian medullary tissue is as safe as cortical tissue to transplant in lymphoma patients. Indeed, the risk of cancer cell reintroduction with ovarian autograft is present in haematological cancer (Meirow et al., 2008).

Conclusions

The emerging technology of ovarian cryopreservation followed by transplantation can be a useful option for fertility preservation in cancer patients. Although transplantation of frozen–thawed ovarian tissue has been successful for restoring fertility and ovarian function, the clinical efficacy and practicability are still uncertain. Due to the limited life span of ovarian tissue grafts, whole ovary transplantation by vascular anastomosis is being explored as an alternative.

To date, no significant advantage has been proven of transplanting the whole intact ovary rather than only ovarian tissue, whereas the risks involved with whole ovary transplantation with vascular anastomosis (especially after freezing and thawing) have been shown to be higher than expected. The safety and efficacy of the procedure should be proven before further clinical applications. Indeed, premature and indiscriminate use of this technology can be harmful and unethical. Nevertheless, efforts to perfect the technology of ovarian cryopreservation and transplantation (with or without vascular anastomosis) should continue, but with extreme caution.

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