

DEBATE

Ovarian tissue banking for cancer patients

To do or not to do?

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A large proportion of childhood and young cancer patients will lose their fertility after aggressive cancer therapy because of the gonadotoxicities of chemotherapy and radiotherapy. One of the strategies to preserve fertility for those facing premature ovarian failure is ovarian tissue banking. We are seeing a growing enthusiasm about this emerging technology. At the same time, scepticism is prevalent, as the efficacy of ovarian tissue banking for fertility restoration in humans has not yet been proved. However, there is ample evidence of its efficacy in animal studies. Ovarian tissue banking requires further investigations and should be considered experimental in humans at present.

Key words: cancer/cryopreservation/fertility preservation/ovarian tissue/transplantation

Introduction

Recently, there has been a surge of interest in ovarian tissue banking worldwide. The human desire to freeze ovaries can be traced back 200 years, although the technique was not successful until the 1950s when the discovery of cryoprotective agents was made. The modern application of ovarian freezing as a strategy to preserve fertility in women with cancer is new, and there are unknowns and doubts about the efficacy of this emerging technology. Some people think that it is too early to offer ovarian tissue banking as a fertility conservation option to cancer patients, as there is no report of pregnancy with this technology in humans yet. There are even opponents of ovarian tissue cryopreservation and transplantation because of the risk of cancer cell transmission. However, it is logical to explore the current status of ovarian tissue banking for fertility conservation before making any conclusions.

Why should we offer ovarian banking to women and children with cancer?

Intensive modern cancer treatment has resulted in an increased number of long-term survivors. For those young cancer survivors, later effects of treatment on quality of life, in particular fertility, can be a very important issue. Unfortunately, high doses of chemotherapy and radiotherapy are gonadotoxic and a large proportion of childhood and young cancer patients will lose their fertility after cancer therapy. In men, sperm banking before cancer treatment can preserve fertility. To date, there is no good safeguard, which is comparable with sperm banking, for female cancer patients. Embryo freezing can be offered prior to cancer treatment, but it

is only an option for patients who have a partner or are willing to accept fertilization by donor sperm. Cryopreservation of oocytes does not require a partner, but it has its own limitations (Kim *et al.*, 2001a). Obviously, neither embryo nor oocyte freezing can be offered to pre-pubertal girls. These two strategies can delay cancer treatment, which is not acceptable to many cancer patients.

An emerging strategy, ovarian tissue banking, can store thousands of immature oocytes effectively. Although restoration of fertility using banked ovarian tissue in humans has not yet been demonstrated, animal studies (Gosden *et al.*, 1994b) as well as on going human studies, are supporting the validity of ovarian tissue banking. If we do not recommend ovarian tissue banking now, many young women with cancer will lose their chance of fertility restoration after aggressive cancer treatment. One may argue if it is ethical to expose the young woman with cancer to surgical risks of laparoscopic oophorectomy or ovarian biopsy for ovarian banking. However, the risk of bleeding and infection with oocyte retrieval in cancer patients is no less than that of laparoscopic surgery. Most importantly, ovarian tissue banking does not delay cancer treatment, unlike oocyte or embryo cryopreservation.

How to collect ovarian tissue?

In most cases, ovarian tissue can be collected using a laparoscopic technique, either laparoscopic oophorectomy or multiple ovarian biopsies (Meirow *et al.*, 1999). For an experienced laparoscopic surgeon, there would be little difference between these two procedures in terms of either operation time or the degree of surgical difficulty. Although there may be

a slight increase in surgical risks with oophorectomy, the benefit of storing a large amount of ovarian tissue can justify removing a whole ovary. In fact, a significant follicular loss occurs with freezing, thawing, and grafting in particular. We do not know how well and how long a given frozen–thawed ovarian section will function after transplantation in humans. For the time being, storing a relatively large amount of ovarian cortex (after processing into thin slices of <2 mm thickness) with unilateral oophorectomy would be a sensible and secure way to preserve fertility.

What is the current status of ovarian cryopreservation?

Although ovarian tissue cryopreservation has been quite successful [>70% survival of primordial follicles after freezing and thawing (Newton *et al.*, 1996)], it requires further improvement to enhance its efficacy. Slow freezing using an automated programmable freezer after equilibration in a cryoprotective agent (1.5mol/l DMSO or propanediol) is a well-established method for ovarian tissue cryopreservation. Vitrification of ovarian tissue also appears to be an effective freezing technique, but it requires further investigation. The challenge in cryotechnology is how to minimize cryoinjuries, especially when thawing bulky tissue. Indeed, significant freezing injury can occur during the thawing phase because of changes in the composition of the surrounding milieu, possibly mediated by leakage of the plasma membrane and re-growth of ice crystals (Kim *et al.*, 2001a).

Is ovarian transplantation a practical strategy?

The main problem of ovarian banking is how to restore ovarian function with banked ovarian tissue. The development of an effective culture system to grow and mature oocytes stored in ovarian tissue *in vitro* can be most desirable, but unrealistic at present. Therefore, ovarian transplantation appears to be a practical strategy to restore ovarian function. Autotransplantation of ovarian tissue in rodents as well as large animals has proved its efficacy in restoring fertility (Parrot *et al.*, 1960; Gosden *et al.*, 1994b; Szein *et al.*, 1998). Recently, there are reports of restoration of endocrine function and maturation of follicles after autotransplantation of either fresh or frozen–thawed human ovarian tissues (Oktay *et al.*, 2001; Radford *et al.*, 2001).

The main concern of autotransplantation in cancer patients is the possibility of cancer cell transmission. Although our recent study demonstrated the safety of ovarian transplantation in lymphoma patients (Kim *et al.*, 2001b), it is not known whether ovarian transplantation is safe for women with other cancers. Indeed, autotransplantation of ovarian tissue should not be considered if there is a significant risk of ovarian metastasis. The risk of cancer cell transmission depends on the disease type, stage and the mass of malignant cells transferred. Ovarian involvement in Wilm's tumour, Hodgkin's lymphoma, osteosarcoma and squamous cell cervical carcinoma is very rare (Young and Scully, 1994), but systemic and hematogeneous malignancies such as leukaemias carry a high risk of ovarian metastasis. Ovarian autotransplantation in breast cancer

patients is not safe, as the frequency of ovarian metastasis during the course of breast cancer is 13–38% (Gagnon and Tetu, 1989; Perrotin *et al.*, 2001). The clinical application of ovarian autotransplantation will be safer and more practical if reliable screening methods to detect cancer cells in the stored ovarian tissue are developed.

Xenotransplantation can provide an alternative to autotransplantation for women with cancer. In fact, xenotransplantation can eliminate the worry of cancer cell transmission. It has been already demonstrated that grafting of ovarian tissue from cat, sheep, and monkey to immunodeficient mice could support follicular development up to the antral stage (Gosden *et al.*, 1994a; Candy *et al.*, 1995). The same results were obtained by xenografting human ovarian tissue to immunodeficient mice (Weissman *et al.*, 1999). Furthermore, ovulatory capacity of the follicles grown in xenografts was evidenced by observing the formation of morphologically normal corpora lutea and the ovulatory concentrations of serum progesterone in host animals (Kim *et al.*, 2002). The ethical concerns and safety issues associated with growing human follicles to maturity in animal hosts should be resolved before the clinical application of this technique.

What should we do for successful transplantation of ovarian tissue?

The most crucial factor influencing tissue survival is the degree of ischaemic-reperfusion injury after transplantation. In fact, more primordial follicles die of ischaemia than of freezing–thawing injury. The survival of primordial follicles after transplantation ranges between 5–50% (Aubard *et al.*, 1999; Baird *et al.*, 1999). Ovarian tissue is endowed with abundant genes for angiogenic factors. Even with this physiologic advantage, the problem of ovarian transplantation without vascular anastomosis is still hypoxic tissue damage that occurs while waiting for revascularization which takes >48 h. Therefore, it is necessary to find a way to facilitate angiogenesis or minimize hypoxia after transplantation.

It could be speculated that antioxidant treatment might protect the ovarian graft from hypoxic injuries, as hypoxia induces the generation of reactive oxygen species (ROS). In fact, our unpublished study using bovine ovarian tissue showed the decrease of apoptosis in the ascorbic acid (antioxidant) treated group. Ultimately, the whole ovary transplantation by vascular anastomosis will be a solution for this problem, given that the technology to freeze the intact human ovary can be developed.

Conclusions

Ovarian tissue banking is considered as a valuable fertility conservation option for cancer patients lately. However, the clinical practicality of ovarian tissue banking followed by ovarian transplantation is still in doubt, as there has been no pregnancy in humans with this technique. Furthermore, the risk of cancer cell transmission is of great concern. In spite of scepticism by some people, there is good evidence to suggest that cryobanking of ovarian tissue will eventually provide a

way of conserving human fertility. The clear clinical benefits of ovarian tissue banking will be proved when restoration of fertility is demonstrated in humans. Until then, ovarian tissue banking should be considered experimental in humans.

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