QOL after childhood cancer therapy -Cutting-edge researches on fertility preservation

Collaboration
Emergency care * Reproductive technique * Organ transplantation

Temporary ovarian transplantation for iatrogenic sterility

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Abstract

Many cancer patients become infertile after treatment with anticancer drugs or radiotherapy. To restore fertility, cryopreservation of sperm, ova/fertilized ova, or ovarian tissue is considered as one of the available options; however, this method gives very low pregnancy rate due to cellular injury caused by freezing. Since it is impossible to collect sperm or ova prior to sexual maturity, there is no other treatment approach that can be applied in the case of children with cancer. In this paper, we propose a new transplantation procedure using rat models in which the function of the reproductive organs is preserved by temporarily transplanting them between living donors and recipients, without the requirement for cryopreservation of the ovarian tissue. These procedures are based on the plastic surgery techniques that are aimed at restoration of amputated limbs. We removed the ovary from a recipient rat, temporarily transplanted it into a recipient rat, and finally retransplanted into the donor; this subsequently resulted in a successful natural pregnancy and delivery. Microscopic observation revealed that most ova that were transplanted to the ovary by vascular anastomosis survived. In addition, femoral bone mineral density analysis confirmed increased bone density compared with that measured prior to the ovarian transplantation. Furthermore, since mammals possess a pair of ovaries, the procedure developed in this study can also be applied to fields other than medicine. For example, the reproductive efficiency of animals after menopause can be increased through reproductive organ transplantation, which can be achieved by preservation of one of these ovaries for transplantation.

Keyword
Super-Microsurgery, Heterotopic transplantation, Evasive transplantation, Fertility preservation

In recent years, the development of cancer therapy has markedly increased survival rates of pediatric cancer patients. However, one of the adverse effects of this therapy is the eventual decline in the reproductive function of the cancer patients. The increased survival rate of cancer patients after therapy has gradually changed the attitude toward their sexuality. Malignancy patients can now hope for marriage, pregnancy, and childbirth after successful treatment. In regards to male patients, particularly in the case of sexually mature boys, sperms can be preserved by temporarily transplanting them between living donors and recipients, without the requirement for freezing. Since it is impossible to collect sperm or ova prior to sexual maturity, there is no other treatment approach that can be applied in the case of children with cancer. In this paper, we propose a new transplantation procedure using rat models in which the function of the reproductive organs is preserved by temporarily transplanting them between living donors and recipients, without the requirement for cryopreservation of the ovarian tissue. These procedures are based on the plastic surgery techniques that are aimed at restoration of amputated limbs. We removed the ovary from a recipient rat, temporarily transplanted it into a recipient rat, and finally retransplanted into the donor; this subsequently resulted in a successful natural pregnancy and delivery. Microscopic observation revealed that most ova that were transplanted to the ovary by vascular anastomosis survived. In addition, femoral bone mineral density analysis confirmed increased bone density compared with that measured prior to the ovarian transplantation. Furthermore, since mammals possess a pair of ovaries, the procedure developed in this study can also be applied to fields other than medicine. For example, the reproductive efficiency of animals after menopause can be increased through reproductive organ transplantation, which can be achieved by preservation of one of these ovaries for transplantation.

In the light of this situation, we propose a procedure in which the ovaries of a patient are temporarily transplanted into another person (recipient), and subsequently retransplanted to the first patient by normal ovarian transplantation procedure after cancer treatment; this can avoid the need of cutting and freezing the ovary and the consequent ischemic injuries to the ovary. This approach, namely, “temporary ovarian transplantation,” was developed on the basis of plastic surgery techniques aimed at restoration of amputated limbs. In the latter, seriously injured limbs are amputated and temporarily grafted onto an uninjured site by conventional methods (heterotopic transplantation) to maintain blood flow; these limbs are retransplanted to the original site after treatment of the injury (Figure 1). This procedure involves vascular anastomosis through super-microsurgery.

Temporary transplantation of a reproductive organ between a living donor and a recipient is a relatively new concept of preservation of organ function. For reconstruction of reproductive function without threatening the life of the patient and for minimizing the risks associated with pregnancy and delivery after transplantation, it is important that transplantation is considered only as a temporary measure. We have thus far investigated different methods of preservation of reproductive organs in an unfrozen state, and in addition,
developed freezing techniques. In order to make these techniques more efficient, we believe that we should utilize conventional transplantation approaches that have been around for more than 100 years.

Figure 1. What is temporary heterotopic transplantation?
An example of heterotopic autotransplantation. A 53-year-old male patient who had his left hand amputated with an electrically-powered saw while he was working [A]. Since he was unconscious from head banging, the affected left hand was temporarily anastomosed to the dorsalis pedis artery and vein (indicated by a white arrow) under local anesthesia on the day of the accident [B]. Retransplantation was performed after recovery from coma, or 14 h after the injury [C].

Figure 2. A baby rat born after temporary ovarian transplantation
The recipient rat was temporarily transplanted with the relevant ovaries that were later retransplanted to the donor rat. The donor rat was then naturally mated with a male rat, resulting in the delivery of normal rat offsprings. Both the recipient rat and the donor rat were in good condition, suggesting that temporary ovarian transplantation can be one of the options of preserving the fertility of the affected individuals.

Figure 3. Ovarian tissues of the vascular anastomosis group and non-vascular anastomosis group
A: Heterotopic ovarian transplantation was performed in the groin of a rat and the ovarian artery (0.3 mm) and vein (0.9 mm) were anastomosed to the subcutaneous trunk artery and vein. Histological evaluation of the ovaries of rats in the ovarian transplantation with vascular anastomosis group and those in the ovarian transplantation without vascular anastomosis group was performed after HE staining. B: After transplantation to the recipient rat, the vascular anastomosis group showed the survival of almost all follicles (× 100 magnification). C: The central part of the ovary was shown to be necrotic in the non-vascular anastomosis group with only few surviving follicles (× 100 magnification). D: Most follicles survived even after retransplantation to the donor rat (× 100 magnification).
Ovarian Transplantation

Bilateral ovariectomy was performed on a 15-week-old F344/jc1 rat via a ventral midline abdominal incision under anesthesia. The ovarian artery and vein of a donor rat were detached up to the renal artery, and the blood vessels were identified; no preservation solutions were used for perfusing the uterine region, and both the ovaries were harvested along with a portion of the uterine horn. While the right ovary was discarded, the left ovary and the blood vessels were transplanted to a 15-week-old F344/jc1 recipient rat, which was administered cyclosporine, an immunosuppressive agent. Specifically, the artery and vein of the left ovary were anastomosed to the femoral artery and vein of the recipient rat, and the ovary and the portion of uterine horn were placed in the abdominal cavity. In the 6th month after transplantation, the grafted ovary and the portion of uterine horn were removed together with the blood vessels that were to be attached to the femoral artery and the vein of the donor rat by end-to-end anastomosis.

Discussion

The results of our study suggested that temporary ovarian transplantation is an innovative method that permits organ preservation without affecting the ovary. Histological analysis revealed that the ovarian structure was well maintained even after transplantation. These findings indicate that our method averts the potential risks associated with the clinical applications of the procedure and facilitates endocrine function recovery or fertility preservation, which are dependent on the follicles in a small part of the ovary even though transplantation of frozen ovaries has been performed in animals or human beings. Since the fragments ovaries become non-functional within a year, they need to be retransplanted as soon as possible; this method permits preservation of all ovarian functions as it is assumed on whole-ovary transplant, and requires no freezing process. While preservation of ovarian function by ovarian transplantation has been clinically performed (autotransplantation) thus far⁹, the reproductive dysfunction after TBI (total body irradiation) is inevitable.

Furthermore, the requirement of a healthy recipient, who has to be treated with immunosuppressive agent, for the preservation of ovaries poses an ethical problem. Nevertheless,
since transplantation is temporary or short-term (a couple of years), treatment with an immunosuppressive agent should not be a cause of major concern. In addition, in case of immunological rejection due to subcutaneous implant or immunosuppression-related side effects, the transplanted ovary can be removed immediately. Studies have been conducted on immunosuppression-related side effects, the transplanted ovary immunological rejection due to subcutaneous implant or not be a cause of major concern. In addition, in case of years, treatment with an immunosuppressive agent should be applied in fields other than medical care. Since mammals possess a pair of ovaries, 1 of them can be used for transplantation. Hence, the reproductive efficiency of domestic animals or race horses is twice that of others, and the preserved ovaries can be transplanted even after menopause. On similar grounds, reproductive organ transplantation has the potential to serve as an effective technique in the field of agricultural science, and can be applied for breeding or conservation of endangered species or superior domestic animals or race horses. This technique also marks the beginning of the modern age in cancer treatment.

<Issue 1: Ethical problems>
Is it ethical to administer immunosuppressive agent to a healthy donor [patient’s mother]?

<Issue 2: Adverse effects, teratogenicity, etc., that may arise due to treatment with an immunosuppressive agent>
Does a donor experience any side effects after transplantation of an extra ovary?

Does treatment with an immunosuppressive agent pose any risk of teratogenicity to the recipient?

Are the immature ovaries affected by this treatment?

<Issue 3: Risk of transplantation between a mother and a daughter>
In what manner are the transplanted ovaries, which contain the tissues of both the donor and the recipient, retransplanted to the recipient?

What are the effects on the recipient if she possesses 3 ovaries?

How can the risk of carcinomatous implants be averted?

### References


### Website References

- Children’s Cancer Association of Japan
  http://www.ccaj-found.or.jp/english/
- University of Tokyo, Academic Collaborations Society
  http://gakujutu.umin.jp/English/

**Conclusion**