—Mini Review—

Cryopreservation of the Ovary

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Abstract: *The removal, cryopreservation, and subsequent reimplantation of ovaries would make it possible to treat a young cancer patient and improve her quality of life by preserving her fertility. The current technology requires cutting the ovary into pieces before freezing and does not support preservation of the whole ovary. The ovary has a complex endocrinologic function. It is composed of cells of different form and character and contains oocytes at various stages of development. Successful cryopreservation, transplantation, and functional rehabilitation of the whole ovary would have broad significance, not only for ovaries but also for other organs such as the liver, kidney, and heart. Ovarian cryopreservation technology would lead the way to the establishment of a biological bank for frozen internal organs.*

Key words: *Cryopreservation, Ovary, Transplantation*

Introduction

Cryopreservation of living cells is an established technology, and the cell banking system provides a source of materials for all aspects of medical research. Clinically, the freezing of mature unfertilized eggs and fertilized eggs has been widely applied to human fertility treatments. What is the difference between freezing ovaries and freezing cells, oocytes, and preimplantation embryos? The difference is the size of the sample. Internal organs are composed of various tissues, and tissues are formed by cells. The egg is the largest cell in the body, but the internal organs are much larger. The size of the sample for freezing affects such factors as temperature change, infiltration of the cryoprotectant, and generation of ice crystals. Recent advances in technology have increased the opportunity to freeze whole ovaries. However, there are still problems to be solved. Here we discuss the current state of the technology for the cryopreservation of whole ovaries.

Received: July 14, 2010
Accepted: July 22, 2010
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Utility of Ovary Freezing

Cryopreservation of the ovary is useful for preserving resources in research using laboratory animals as disease models and for transgenic studies. Clinically, this technology is shifting from the research area to practical uses for maintenance of fertility and improving the quality of life of cancer patients [1–11]. It has even been applied to patients with Turner syndrome, whose ovarian follicles are lost with age. In younger patients, ovary freezing could be used when the vaginal collection of eggs is difficult. At present, human fertility is supported by the use of assisted reproductive techniques such as *in vitro* fertilization and intracytoplasmic sperm injection into mature or immature oocytes. The probability of achieving conception depends on the method of egg collection before the ovary is frozen. Therefore, ovary freezing will expand the range of current applications in the medical technology.

Current State of Ovary Cryopreservation

Ovary cryopreservation was developed using animals [12–16] such as mice and monkeys. Ovary freezing is believed to be a useful means for preserving reproductive cells, but the technology to achieve conception is not yet at the practical stage. The first child to be born from the transplantation of frozen-thawed sliced ovaries was reported in 2004 [3, 4]. Since then, human births resulting from the use of this technology have been widely reported [5–7]. For cancer patients, the ovaries are removed before the

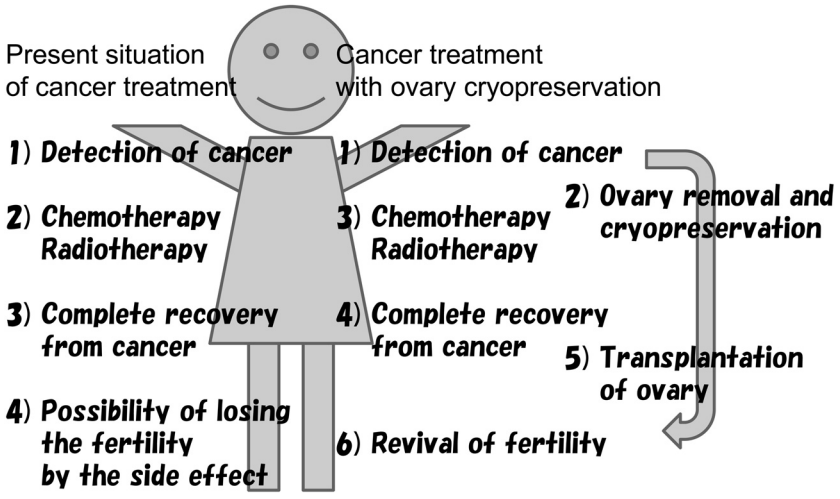

Fig. 1. Example of the application of ovary cryopreservation. The fertility of a cancer patient may be lost due to the side-effects of the treatment of the cancer cells with chemotherapy and radiotherapy (Left side). Before the treatment of the cancer, the ovary can be removed and preserved (Right side).

start of cancer treatment, and the frozen-thawed ovaries are retransplanted to the patient when the cancer has been successfully treated, because there is a possibility that the ovarian germ cells may undergo damage along with the cancer cells during treatment. One of the new cancer treatments preserves the fertility of the patient, but this technique is complicated (Fig. 1).

The current method for ovary freezing involves slowly freezing slices of the ovary. This method was devised to improve the success rate of freezing because the whole ovary is too large to be frozen using current methods. It is common to use a cryoprotectant for cell freezing. By slicing off a large piece of ovary tissue or cutting it into smaller pieces, the effect of the cryoprotectant is known. However, there can be physiological and biological problems with slicing or cutting the ovary. The organ slice begins to lose cells at the contact site of the slice with the screen insert, and thereby reduces organ slice architectural stability and viability. We believe that many problems can be avoided if it were possible to freeze the whole ovary, and into whole ovary preservation has already begun.

Theme for the Future

Thinly slicing the ovary or cutting it into small pieces is an excellent way for the cryoprotectant to infiltrate the specimen and for an even temperature to be maintained. However, the number of ovarian follicles that exist in a cut ovary (10 mm × 10 mm × 1 mm) is thought to be limited, and many of them can be lost during the process of freezing, thawing and transplantation. It seems that small ovarian follicles (such as a primordial ovarian follicle) can survive. Therefore, it is currently necessary to wait for several months to confirm the functionality of a transplanted ovary. Furthermore, the long-term maintenance of ovarian function cannot be guaranteed. It is necessary to analyze in detail the living cells in the thawed ovary. Cells (oocytes) at various stages and cells with endocrinologic function exist in the ovary. The state of each cell cannot always be determined. Moreover, it will be necessary to verify the effect of cryoprotectant and its side-effects after transplantation.

It is not known why a transplanted ovary sometimes does not function. Therefore, the problems of cryopreservation cannot be solved by focusing on the freezing technology alone. It is also necessary to perfect the thawing method. In the thawing method, it is extremely difficult to thaw both the inside and the outside of large internal organs under the same conditions. It is difficult to achieve success according to the fundamental principles of physics in freezing theory, even though the theory has been clarified [17–23]. When one does not achieve an excellent result by analyzing a frozen-thawed organ transplantation sample, it should be considered that there should be problems in both the freezing and the thawing

technology. Thus, the further development of thawing technology is an important area of research.

Moreover, the implantation technique cannot be disregarded. It is necessary to make blood circulate within the ovary several hours or a few days after transplantation of the ovary. If the nutritional content of the cells is not supplied with blood, an individual cell cannot survive at body temperature. Ideally, each ovary is frozen with a blood vessel, and the blood vessel is sutured during transplantation. We are attempting to improve the method of cryopreservation of blood vessels to solve this problem.

In one clinical application of cryopreservation, the ovaries of female cancer patients are removed and frozen before cancer treatment begins, and then the ovary is transplanted after the cancer has been successfully treated. There will be no cancer cells in the ovaries, but because of the possibility of minimal residual disease (MRD), it is necessary to assess the transplant carefully. A new technology for MRD detection needs to be developed [24, 25]. Furthermore, an ovum, embryo or ovary should be selected for cryopreservation according to the patient's age and the degree of damage to the ovary, because of the close relationship between the age of a woman and the degree of ovarian function.

It is necessary to select the appropriate treatment for each patient who desires a natural pregnancy, such as assisted reproductive techniques involving ovary removal on only one side, whole ovary removal, or partial excision. Moreover, data should be accumulated about each technique to improve the survival rate of the ovary after transplantation and the timing of retransplantation to maintain ovarian function at the site of the graft.

Upgrade of Technology and the Importance of Education

Theoretically, it is effective to freeze the whole ovary and to keep the ovary function long time when transplanting an ovary. If the loss of ovarian follicles can be reduced to the minimum, ovarian function should be maintained for a long time. To achieve this, it is necessary to develop freezing and thawing technologies for the whole ovary and to solve the problem of thrombosis during vascular anastomosis. The rate of pregnancy after such procedures is extremely low, although pregnancies have been reported in animals. However, live births after transplantation of the whole ovary have not been reported in humans [26].

Cryoprotectants cannot work properly on the whole ovary. However, the damage from ice crystals in cells can be reduced by slightly magnetizing the whole ovary during freezing, without a cryoprotectant. The following conclusions are possible: (i) a cryoprotectant is not needed, (ii) thrombosis can be prevented, and (iii) large growing follicles can survive. If this magnetization technique could be established, it would be possible to apply it not only to the ovary but also to other internal organs. This technology may even be useful in the field of organ transplantation.

Although research on the cryopreservation of internal organs started only recently, this technology should improve the ability to treat cancer in young patients and their quality of life after recovery [27, 28]. Medical researchers and physicians in various fields should cooperate in the development of this technology. We strongly encourage supplying accurate information about the technology not only to medical personnel but also to the general public.

Possible Applications of Cryopreservation Technology in Other Fields, and Future Perspectives

The most important goal of cryopreservation technology is to maintain the function of the cell after freeze-thawing. The ovary is one of the sources of female reproductive cells and has an endocrinologic function. In other words, it is a complex internal organ that contains cells of various kinds and at various stages of development. Once the freezing of the ovary becomes feasible and the return to ovarian function after transplantation is certain, it may also be possible to freeze other internal organs such as the heart, liver, and kidneys. As we know that the cell-mediated immune response molecule, the major histocompatibility complex (MHC), varies greatly between individuals and mismatch of MHC antigen is an important factor in the acute rejection of the transplanted tissues. If an information bank of MHC antigen for frozen internal organs used in organ transplantation can be successfully developed, medical treatment with organ transplants could be greatly improved, since it is thought that a frozen internal organ banking system could serve the entire world (Fig. 2). In addition, the utility of the umbilical funiculus, including stem cells, is parallel to that of bone marrow stem cells. If freezing the umbilical funiculus were to become possible, the number of stem cells preserved would increase. Technological development might also enable the cryopreservation of blood.

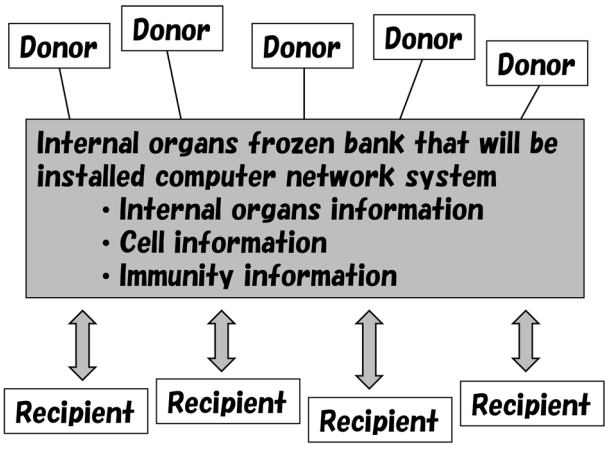

Fig. 2. Schematic diagram of an internal organ cryobank. Time can be saved in the transportation of internal organs and the matching of the donor and the recipient. The establishment of an organ cryobank would help to meet many of the challenges faced in transplantation of an organ between a donor and a recipient living elsewhere in the world.

Ovary cryopreservation technology contributes greatly to both laboratory animal research and clinical applications. Further advancement of organ cryopreservation technology is expected to occur through research into the freezing of different organs in the near future.

Conclusion

The technology of ovary cryopreservation has progressed and has opened many possible doors. This technology will give hope to young women with cancer. Moreover, existing research suggests the possibility of constructing a bank for frozen internal organs.

Acknowledgements

The authors would like to thank Dr. Lubna Yasmin, researcher, Tsukuba Primate Research Center, National Institute of Biomedical Innovation for valuable assistance in preparing this manuscript.

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