Collaboration **Reconstructive surgery * Transplantation surgery**

Uterus transplantation and ovarian cryopreservation for fertility reconstruction in female genital cancer patients

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(Received 5 May 2009; accepted 8 June 2009)

Abstract

Recently, a therapy aimed to preserve fertility has been developed for patients with female genital cancer. However, carcinoma excision needs to be performed for cases of advanced disease stage at the expense of fertility. We have been conducting an ovarian cryopreservation study and uterus transplant study under immunosuppression in an attempt to reconstruct fertility in patients with a radical hysterectomy due to advanced disease. It may seem to be too early to expect clinical application of a uterus transplant study at this stage of development because many ethical and legal problems remain to be solved. However, we believe it is very important to raise the possibility for those patients undergoing radical hysterectomy to have babies by cryopreserving a part of the removed ovary so that IVM-IVF (in vitro maturation-in vitro fertilization) technique may be adopted in the future when the technology advances to the point of achieving a mature ovum from an oögonium.

We aim at safe fertility reconstruction in patients who had a hysterectomy due to uterine cancer by establishing a new uterus transplant based on an effort to integrate Super-Microsurgery, an advanced vascular anastomosis technique in the plastic surgery and reconstructive surgery field, and organ transplantation under immune tolerance, an advance in the transplantation surgery field. Considering studies of surrogate mothers and uterus transplants, cryopreservation of part of removed ovarian tissue (not used for pathological diagnosis) in patients with female genital cancer can be very important. We present the following report on the development of uterus transplant experiment model of pigs.

Keyword

Super-Microsurgery, Ovarian transplantation, Immune tolerance

Introduction

Assisted reproductive technology (ART) has developed in Japan, and advanced reproductive technology was involved in 1.8% of all offspring¹⁾. However, such therapy does not suit infertility caused by a problem in the uterus. This type of infertility can be divided into the congenital (dysplasia such as Müllerian duct hypoplasia and aplasia and Mayer-Rokitansky-Küster involves deficits of the uterus and the vagina) and acquired (hysterectomy as a treatment for uterus myoma, endometrial adhesion, uterine body cancer, and uterine cervix cancer) types²⁾. This paper specifically discusses infertility resulting from hysterectomy as a treatment for uterine cancer and ethical problems with regard to uterus transplants and surrogate mothers.

There is a recent report arguing that uterine cervix cancer is caused by a sexually transmitted disease associated with human papillomavirus (HPV)³⁾, and the importance of preventative medical examination for uterine cervix cancer has been proposed. The testing of HPV has become available recently due to the advances in testing technology. The diagnosis of uterine cervix cancer can maintain a significantly high accuracy with HPV testing and cytodiagnosis to find in the

stage prior to canceration (severe dysplasia). Treatment of the disease may be relatively simple at such an early disease stage, which involves a treatment called conization to remove only uterine cervix by electrocautery or laser. Most cases of uterine cervical cancer can be completely cured with this single treatment without removing the uterus. In addition, this treatment does not influence pregnancy and childbirth. Patients with advanced stage female genital cancer, however, cannot use this treatment because cancer therapy should be prioritized and requires other therapies, such as radical hysterectomy or bilateral ovariectomy.

We have been conducting research on tissue cryopreservation of a removed ovary from the standpoint of the future fertility reconstruction (Figure 2). We have also started working on the basic research of uterus transplants under immune tolerance with the aim of reconstructing the uterus.

Ovarian cryopreservation after hysterectomy has not been performed so far because of its association with surrogate mothers in Japan. Surrogate mothers, in principle, are not legally recognized in Japan, and the Science Council of Japan proposes the following, see the website for details (<http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-t56-1e.pdf>).

Propositions have been put forward regarding the issues

about ART centering on surrogate gestation at the meeting of the Science Council of Japan in April 2008: (1) it is advisable to legally regulate and principally prohibit surrogate gestation; (2) physicians performing surrogate gestation for profit, agents, and clients are subject to punishment; (3) clinical studies in women with congenital absence of the uterus and women who had a hysterectomy as treatment may be conducted under close supervision; (4) in consideration of surrogate gestation, establish a public administrative authority consisting of specialist in medicine, welfare, law, and counseling and this authority will either approve by advocating legal reform or issue a denial of the practice after careful deliberation; (5) the child born by surrogate gestation is recognized as a child of the surrogate; and (6) the parent-child relationship between the child and the client couple is determined by the adoptive or special adoptive relationship. With regard to various issues relating to bioethics, the establishment of a separate public research institution and public standing committee was recommended in order to deal with the issues. With the first priority given to the well being of the child, the child's right to know his/her origin, egg donation and gestation from postmortem frozen sperm retrieval were recognized as important investigative subjects for the future.

History of uterus transplant

Fallopian tube transplant and uterus transplant started in the 1960s and 1970s. There are two major problems with these transplantations, which includes vascular anastomosis techniques and immunorejection. In order to address these

problems, autotransplantation was implemented first. Uterus transplant experiments were conducted by O'Leary et al.⁴⁾ using dogs in 1969 and Scott et al.⁵⁾ using Macaque monkey in 1971, and the results showed success rates of 80% or more in both autotransplantation studies. Births were reported in autotransplantation of the uterus and ovaries in dogs^{6, 7)}, and a successful case of allotransplantation/xenotransplantation was reported in the use of immunosuppressive drugs⁸⁾.

Prior to these studies, uterus transplants had been performed accompanied with the uterine tube, and the uterus was considered an appendage to the uterine tube in the transplant procedure⁵⁾. A subsequent development of IVF-ET (in vitro fertilization-embryo transfer) technique, therefore, significantly reduced the importance of the fallopian tube transplant, and this research field temporarily declined, which was facilitated by the issue concerning immunosuppressive drugs. However, a series of advances, such as the development of the microvascular anastomosis technique, elucidation of the mechanism, and immunosuppressive drugs, have substantially contributed to the development of the research. Racho El-Akouri et al. conducted a study of uterus transplants in mice and successfully achieved pregnancy from a transplanted uterus by in vitro fertilization and embryo transfer⁹⁾. In Japan, Nishida reported fallopian tube and uterus transplant experiments in dogs and baboons in 1990 and 2000, respectively, and asserted that fallopian tube and uterus transplants are technically possible in humans¹⁰⁾.

Figure 1. Pig uterus transplant

A surgery where the uterus is being removed by two surgeons using an endoscope (left). Four port insertion openings on the abdominal wall and two inguinal incisions are made in a pig under general anesthesia. The length of the inguinal incision should be long enough to allow uterus transplant, which is approximately 5 cm. The pig's uterus is bicornuate uterus, and the uterine horn was exteriorized from the inguinal incision to enhance the procedure in the abdominal cavity. The pig's bicornuate uterus was removed from the 5 cm inguinal incision (middle). The ablation of the uterine artery and vein was performed in the abdominal cavity under laparoscope. Alternatively, uterine horn resection could also be performed at the fimbria ovarica with the laparoscope. The uterine artery and vein were removed outside the abdominal cavity after identification with the laparoscope and marked by a ligature (right). Ablation of the uterine artery and vein was subsequently performed based on the ligature mark outside the body. The diameters of the uterine artery and vein were 0.8 mm and 1.0 mm, respectively, which were large enough to perform the vascular anastomosis.

Figure 2. Cryopreservation of ovarian tissue

The photo shows the fragments of ovarian tissue prior to freezing. Currently, IVM-IVF (in vitro maturation-in vitro fertilization) of oögonium from a frozen section of ovary tissue is actively investigated. Oögoniums in the frozen tissue may be used in the future to achieve a mature ovum and fertilized egg.

Figure 3. Anatomical characteristics of primate internal genitalia

The internal genitalia of cynomolgus monkeys are very similar to that of humans. The uterus is single, and the ovarian and fallopian tubes are placed in the same position as those of humans, shown in the figure on the left. Other resemblances are observed where the uterine artery diverges from the internal iliac artery and the uterine vein exits in a reticulate manner.

World's first human uterus transplant

In 2002, the world's first human uterus transplant was reported¹¹⁾. The donor was a 46-year-old woman, and the recipient was a 26-year-old woman who had a hysterectomy due to postpartum hemorrhage six years before; surrogate birth was not allowed for religious reasons in Saudi Arabia where the operation was performed. In the transplant, vascular anastomosis to the external iliac artery was performed utilizing the donor's great saphenous vein to extend the recipient's uterine artery and vein. Acute immunologic rejection occurred nine days after surgery but was successfully treated. Ninety-nine days after surgery, acute thrombus occurred in the uterine artery and vein, by which necrosis was caused in the uterus, and the uterus was removed.

Human renal transplantation under immune tolerance

Premature delivery and abortion and teratogenesis due to immunosuppressive drugs are problems in uterus transplants. In the 50 years of transplant history, more than 15,000 children were born to women who received transplantations, but immunosuppressive drugs entailing the risk of teratogenesis have been reported in animal experiments. Although the immune system of the uterus had been considered different from those of other organs, the findings from animal experiments have indicated no significant difference in the immune system between the uterus and other organs. We believe that it is desirable to avoid the administration of immunosuppressive drugs, especially so when considering the physical strain on the patient.

Kawai et al. a research team from a Massachusetts hospital (USA), reported breakthrough findings in January 2008 that they successfully performed renal transplantation without administering immunosuppressive drugs¹²⁾. An HLA mismatch kidney was used in this transplantation. After the surgery, the dose of immunosuppressive drugs (cyclosporine) was gradually reduced, and administration was not required in four out of five patients 9–14 months after transplantation. The kidneys have been working properly as of today, 2–5 years after transplantation.

A condition where a transplant is functioning without immunosuppressive drugs is called immune tolerance, which has also been reported in other transplantations. While the mechanisms are not completely known, it is generally considered that immune tolerance may be maintained with the coexistence of hemopoietic stem cells from the patient and donor inhibiting the attack on the transplant.

We strive for the possibility of a uterus transplant without immunosuppressive drugs for patients who had a hysterectomy due to cancer therapy in the hope of risk management in labor and psychological protection of the baby. We succeeded in establishing a transplant procedure with a pig uterus, which is relatively similar to that of humans in size, and present the report as follows.

Results

Pig uterus transplant

Vascular anastomosis of the pig uterine artery and vein was performed, and the color of the uterus subsequently appeared good. The diameters of the uterine artery and vein were 0.8 mm and 1.0 mm,

respectively, which were large enough to perform vascular anastomosis (Figure 1).

Primate uterus transplant

The anatomy of the internal genitalia is different between humans and pigs not only in the form of the uterus but also blood stream; therefore, the success of a pig uterus transplant does not necessarily mean success in a human uterus transplant. If a human uterus transplant is assumed, a transplant experiment in primates, which are anatomically closer to humans, should be conducted. We carried out a study to investigate the vascular anatomy of the uterus and ovaries in a cynomolgus monkey (Figure 3). The results indicated that the internal genitalia of cynomolgus monkeys is significantly similar to that of humans, and a uterus transplant is technically possible.

Discussion

Nishida proposed three main problems in uterus transplants that need to be solved in the future. The first problem, regarding the technique, is the low visibility of the vessel for the graft bed in patients after the removal of uterine cancer. In patients who had surgical resection of uterine cancer, the ligation and dissection of the ovarian artery and vein occur in a hysterectomy, which leaves the abdominal cavity adhered. The ablation and development of the vessel for the graft bed have been considered extremely difficult in this condition. The second problem is the necessity of administration of immunosuppressive drugs. It is commonly accepted that immunosuppressive drugs need to be administered during transplant surgery, but the proper type, dose, and period of administration are not known. A related issue may be whether a transplanted uterus can properly adapt to fetus growth in a successful transplant after pregnancy. The third and most important problem is the selection of a uterus

Figure 4. Blood stream of human inferior epigastric artery and vein visualized by MDCT (multidetector-row computed tomography)

Due to the advances in CT (computed tomography) techniques, a detail investigation has become available prior to surgery. Inferior epigastric artery: it originates from the superior or directly posterior to the inguinal ligament and immediately makes an arched superior lateral curve; if the artery originates from directly posterior to the inguinal ligament, it subsequently crosses over this and reaches the dorsal surface of the anterior abdominal wall; it crosses over the deferent duct with a superior lateral concave curvature, runs in an arching line, covered by the peritoneum, with the convex side facing inside while bordering between the lateral and medial inguinal fossa; it reaches an arcuate line inferior to the rectus sheath and communicates with the end of the anterior epigastric artery at the level of the umbilicus after distributing over the rectus abdominis.

donor, seen as a social matter. A related ethical matter can be expressed by the question as to whether the uterus transplant, an organ transplantation, may be performed in order to achieve fertility in “healthy” women and not for lifesaving purposes.

We have a solution for each of the three problems. We propose the use of the deep epigastric artery and vein, nutrient vessels for the rectus abdominis as a solution to the first problem, instead of seeking a vessel for the graft bed in the abdominal cavity where significant adhesion is expected. The deep epigastric artery and vein diverge from the femoral artery and vein and provide nutrients for the rectus abdominis. The vessels have a diameter of approximately 2.0 mm and have been anatomically stable enough for plastic surgery and reconstructive surgery (Figure 4).

Our solution to the second problem is to establish a transplant protocol that does not require immunosuppressive drugs. Kawai et al. reported that they achieved successful human renal transplantation that did not require an immunosuppressive drug by transplanting a small amount of bone marrow prior to the organ transplantation¹²⁾. This approach prevents the body of the recipient from recognizing the organ of the other person by creating a state of microchimerism with two types of hemopoietic stem cells in the recipient's body. We believe that this groundbreaking protocol of organ transplantation under immune tolerance is more likely to lead to successful transplants of the uterus, which is structurally simpler than the kidney.

Our proposition for the third problem is to perform transplantation from patients with gender reassignment surgery. The estimated number of patients with gender identity disorder is 2,500–7,000 in Japan, and 50–100 cases of gender reassignment surgery have been performed per year mainly at Okayama University and Saitama Medical University. Of those cases, female to male (FTM) cases account for 25–50 per year. Transplantation of the discarded uterus in gender reassignment

surgery to patients who lost their uterus does not increase the risks. We believe that this option should be discussed in greater detail.

Conclusion

Uterus transplants involve a number of ethical and legal issues, and there is a long way to go before clinical application. At this stage, we emphasize the importance of freezing the ovaries to keep the option open to having a baby. In doing so, we propose preserving fertility by ovarian cryopreservation of the remaining tissue after pathological diagnosis using the removed ovaries. This treatment can be essential in improving the patient's QOL (quality of life) after cancer therapy.

We recognize that there are issues to be discussed regarding surrogate mothers and uterus transplants but believe it very important to maintain the option to become pregnant and have a child for patients with cancer even if a chance may be quite small.

<Issue 1 Legal and Ethical Issue>

Whose uterus should be transplanted?

<Issue 2 Possibility of mixing cancer cells in cryopreserved ovarian tissue>

The ovary for cryopreservation may contain metastatic cancer cells that were not detected by histopathology.

<Issue 3 Possibility of uterus transplant under immune tolerance>

The transplant under immune tolerance is still being investigated by clinical studies. No clinical case has been reported other than renal transplantation. This requires further investigation to raise the possibility.

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