

Ovarian cryopreservation and transplantation: A preserving fertility procedure

Bram Pradipta^{1,2}, Rajuddin², and Mohd Andalas²

¹Obstetrics and Gynecology Department – Faculty of Medicine University of Indonesia, Jakarta- Indonesia;

²Obstetrics and Gynecology Department – Faculty of Medicine Syiah Kuala University, Banda Aceh- Indonesia E-mail: bram_p@yahoo.com

Abstract. Cancer is a major health problem in both developed and developing countries. In women, cancer incidence rates increased every year. Developments in treatment modalities and the ability to detect tumours in the early stages increased their survival rate but also raise fertility problems. Those problems are the fertility preservation for patient who have to endure gonadotoxic chemotherapy and or radiation even though they still need their fertility functions. Ovarian cryopreservation and autotransplantation were initially designed to protect and restore reproductive function in patients receiving sterilizing chemotherapy and/or radiotherapy. Other indications including patients undergoing haematopoietic stem cell transplantation, autoimmune diseases and those undergoing oophorectomy for non-cancer conditions. Options in cryopreserved ovarian tissues include autotransplantation and xenotransplantation. An orthotopic site or a heterotopic site can be considered for autotransplantation. Xenotransplantation of human ovarian tissue into immunodeficient animals can prevent immunological rejection. The theoretical advantage of orthotopic grafts is the restoration of normal reproductive function and natural conception after transplantation but application for cancer patients is problematic because of the potential risk of transmission of microscopic metastatic disease. With Xenotransplantation, the possibility of cancer transmission and relapse can be eliminated because cancer cells cannot penetrate the zona pellucida, and some technical difficulties of in vitro growth and maturation of primordial follicles can be bypassed. But it is still unknown whether conditions for the growth and maturation of human oocytes in an animal host are comparable to those in situ and whether animal pathogens can be transmitted to human tissue with it. Ovarian tissue cryopreservation is the fertility preservation option for prepubertal girls and for women who face the high likelihood of diminished ovarian reserve requiring immediate treatment. Its procedure are still within improvement and also in the study of understanding its mechanism. In the future, studies and large clinical trials are still needed to develop better cryoprotectants and cryopreservation protocols and also standardization - optimization transplantation techniques

Keywords: ovarian, transplantation, cryopreservation, fertility

Introduction

Cancer is a major health problem in both developed and developing countries.¹ In women, cancer incidence rates increased every year. Developments in treatment modalities and the ability to detect tumours in the early stages increased survival rate but also raise fertility problems. Those problems are the fertility preservation for patient who have to endure gonadotoxic chemotherapy and or radiation even though they still need their fertility functions.. Many treatments that are administered for childhood and adolescent cancers carry a substantial risk for infertility.² This risk varies according to the presenting pathology and requires preventive treatment. It was estimated 1 in 1,000 adults was a survivor of childhood cancer. Over the last decade, the field of ovarian transplantation and cryopreservation has significantly progressed, becoming applicable in humans. Cryopreservation of embryos is a standard technique for fertility preservation when there is adequate time for ovarian stimulation¹ But this technique requires at least 2 weeks from the beginning of the menstrual cycle, which may not be available to all patients with cancer. Furthermore, embryo cryopreservation requires a partner and ovarian stimulation, both of which are not possible in prepubertal girls.³ Ovarian cryopreservation and autotransplantation are the other method and were initially designed to protect and restore reproductive function in patients receiving sterilizing chemotherapy and/or radiotherapy. Other indications including patients undergoing haematopoietic stem cell transplantation, autoimmune diseases and those undergoing oophorectomy for non-cancer conditions

Indications

Premature ovarian failure (POF) is a wellknown consequence of exposing female gonads to chemotherapeutic drugs. The indications now include not only neoplastic diseases but also non-neoplastic conditions requiring chemotherapy, radiotherapy or haematopoietic stem cell transplantation. Oophorectomy for benign ovarian tumours and for BRCA germline mutations can also be indications.² It was used in treating Hodgkin's disease, breast cancer or during treatment for autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus.² Histological sections of the ovary after exposure to cytotoxic drugs show a spectrum of changes

ranging from decreased numbers of follicles to absent follicles to fibrosis.⁴ The population of women who may potentially require gonadal protection against alkylating agents is quite large. Twenty-five percent of breast cancer is diagnosed in women who are under the age of 50. With the typical regimen of CMF (cyclophosphamide, methotrexate, fluorouracil), two-thirds of women will become amenorrhea. With the AC (doxorubicin, cyclophosphamide) protocol, 34% will be amenorrheic at 3 years. This percentage increases if taxanes are also used with this regimen.

Some Indication for ovarian tissue cryopreservation¹

- Cancer in children
- Breast cancer
- Cervical cancer
- Autoimmune and hematological disease
- Surgery for benign ovarian disease
- Patients receiving pelvic radiation
- Prophylactic oophorectomy

Ovarian cryopreservation

Cryopreservation of ovarian cortex combined with orthotopic transplantation into an irradiated ovary restored fertility in rodents 50 years ago⁵. The ability to preserve oocytes and ovarian tissues in a healthy state for a variable duration gives the patient who will undergo cancer treatment another option to preserve her fertility.^{2, 6-7} A major advantage of ovarian cryopreservation is that this technique does not delay cancer treatment, unlike cryopreservation of embryos or oocytes.⁸ Cryopreservation of oocytes and gonadal tissues (i.e., ovarian and testicular tissue) is a rapidly evolving area in reproductive medicine.⁹ It relies on the principle that the primordial follicles withstand the cryo-toxicity better than the growing follicle. A relatively inactive metabolism, lack of zona pellucida, and metaphase spindle grant this privilege to primordial. In addition, smaller cell size allows faster penetration of cryoprotectants in the oocyte.^{9-10 11} The main factor that may influence the outcome in oocyte cryopreservation is its structural complexity. Oocyte subcellular organelles are far more complex and perhaps more sensitive to thermal injury than preimplantation embryos.²

Xenografting studies of human ovarian tissue

Cortical fragments of human ovarian tissue can be xenotransplanted into T- and B-cell-deficient SCID mice. It was demonstrated that cryopreservation and xenotransplantation did not appear to greatly affect the human primordial/primary follicle ultrastructure. Interestingly, in frozen-thawed xenografts, secondary human ovarian follicles presented a well-preserved ultrastructure, but asynchrony between oocytes and granulosa cell development was detected.¹² GnRH agonist treatment did not prevent primordial follicle depletion after the xenografting of ovarian tissue in SCID mice with or without gonadotropin stimulation.¹³ Furthermore, GnRH α caused an additional loss of follicles if administered during the critical neo-vascularisation period after the transplantation until now its use as a means to use banked ovarian tissue is in question.^{1, 3, 10} Concerns regarding cross-species retroviral infections should also be addressed. With this technique, the possibility of cancer transmission and relapse can be eliminated because cancer cells cannot penetrate the zona pellucida, and some technical difficulties of in vitro growth and maturation of primordial follicles can be bypassed. Additionally, this technique can be applied to patients at high risk for hyperstimulation syndrome (e.g., polycystic ovary syndrome) or to patients for whom hormonal replacement therapy is contraindicated such as those with breast cancer.⁸ It is unknown whether conditions for the growth and maturation of human oocytes in an animal host are comparable to those in situ. It is also of concern that animal pathogens can be transmitted to human tissue with xenografting.^{8, 14-15}

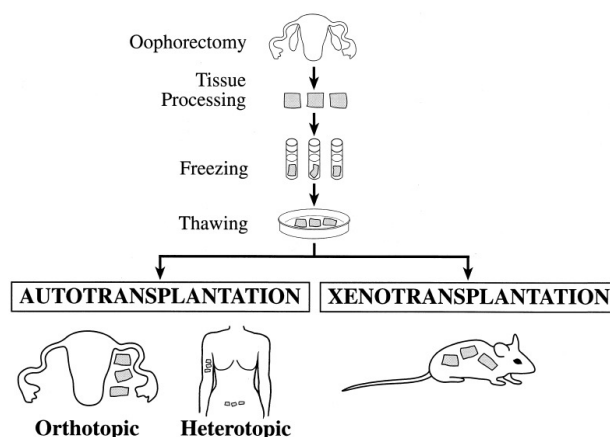


Fig 1. Options for development of immature oocytes in cryopreserved ovarian tissues ⁴

Human Ovarian cortex transplantation

There are two main approaches for autotransplantation of human ovarian tissue. In the heterotopic transplantation, cortical fragments can be grafted subcutaneously at various sites; such as forearm and abdominal wall. ¹⁶In orthotopic transplantation, ovarian cortical fragments are transplanted into its original location, on the remaining ovary or near the infundibulopelvic ligaments or ovarian fossa. ¹

Orthotopic transplantation

The advantage of orthotopic transplantation is that a natural pregnancy can occur; however, the procedure requires abdominal surgery and general anaesthesia. However, orthotopic location is not preferred when the risk of ovarian metastasis is high because tissue monitoring may be more difficult. ¹⁷

Heterotopic transplantation

In this technique, general anaesthesia or abdominal surgery is not required, follicle monitoring and, when necessary, removal of transplanted tissue is easier in a subcutaneous site. Various body sites can be used to graft ovarian pieces, subcutaneous space above the brachioradialis fascia of the forearm or under rectus sheath in lower abdomen. Heterotopic transplantation may be indicated if the pelvis is not suitable for transplantation due to previous radiation or severe scar formation. Furthermore, easy tissue monitoring is an advantage of heterotopic transplantation. There are still numerous challenges to perfecting the heterotopic ovarian transplants as the oocyte maturation process appears to occur differently than in the orthotopic environment. It can also be done through laparoscopic process. ¹⁸⁻²¹

Freezing/ thawing of ovarian cortex may be indicated for adult women who cannot delay the start of chemotherapy, making controlled ovarian stimulation for oocyte or embryo freezing unrealistic. In addition, it is the only option available for prepubertal girls to save their fertility. Two techniques have been reported. The first option is to extract around 50% of one ovary by removing a block of cortical tissue or by removing 5–10 ovarian cortex biopsies with an average volume of around 5 mm³ per biopsy. ²²

It is now well established that adequate penetration of cryoprotectant through the stroma and granulosa cells to the oocytes is required for obtaining satisfactory results. The choice of cryoprotectant with maximum permeation capacity but minimum toxicity and ice crystal formation potential is specific to each cell and tissue type. In the ovary, this choice requires the adequate compromise among the stroma, the follicular cells, and the oocytes. ⁵ Human studies comparing slow-freezing protocols with vitrification of ovarian tissue have produced conflicting results, which may be explained by differences in the protocols and the medium used. However, transmission electron microscopy data suggest that vitrification could be more effective than slow-programmed freezing when cryopreserving ovarian tissue.

Whole ovary cryopreservation

Cryopreservation of whole human ovary is a challenging issue. First, human ovary is larger and more complex than the ovaries of the animals; and second, it may be challenging to devise a cryopreservation protocol that will optimally preserve both the ovarian follicles and

vasculature structures. Several authors demonstrated that cryopreservation of intact human ovary with its vascular pedicle is not associated with any signs of apoptosis or ultrastructural alterations in any cell types. However there has been no case of successful ovarian transplantation with whole-frozen ovarian tissue and no study reported on the functionality of ovaries frozen intact.²³

The Dangers

There are still risks of re-implanting an occult tumour with frozen– thawed ovarian pieces; When there is a high risk of ovarian metastasis, ovarian transplantation for the purposes of future autotransplantation should not be performed. Patients with high-risk cancers either should not be given the option of ovarian autotransplantation or ovarian tissue harvest should be performed after the first round of chemotherapy in order to clear any neoplastic cells residing in the ovary. However, it should be stressed that the ovarian reserve and effectiveness of assisted reproductive technologies diminish with each round of chemotherapy administered.^{1, 24} In all cancer patients, to further minimize the risk of cryopreserving cancer cells with ovarian tissue, multiple biopsies should be taken from the ovary and a thorough histological analysis should be performed. Additionally, when there is a marker, molecular biology techniques as well as immunostaining can be used to detect occult metastasis²⁵

Regardless of the application, the practitioner should have a thorough discussion with the patient regarding all the available options and make it clear that most fertility preservation options are currently experimental.¹ In vitro maturation of primordial follicles and ovarian tissue xenotransplantation may 1 day become common applications in conjunction with the cryopreservation of ovarian tissue. To avoid possible reimplantation of malignant cells, two approaches have been suggested, such as grafting of isolated follicles in an artificial ovary and in vitro maturation of primordial follicles In this setting, a whole ovary cryopreservation has an advantage over cortical strips.⁵

Conclusions

Ovarian tissue cryopreservation is the fertility preservation option for prepubertal girls and for women who face the high likelihood of diminished ovarian reserve requiring immediate treatment. Its procedure are still within improvement and also in the study of understanding its mechanism. In the future , studies and large clinical trials are still needed to develop better cryoprotectants and cryopreservation protocols and also standardization - optimization transplantation techniques

Acknowledgements

Obstetrics and Gynecology Department–Faculty of Medicine Syiah Kuala University, Banda Aceh. Obstetrics and Gynecology Department–Faculty of Medicine University of Indonesia, Jakarta

References

1. Sonmezer M, Oktay K. Orthotopic and heterotopic ovarian tissue transplantation. *Best Practice & Research Clinical Obstetrics and Gynaecology* 24 (2010) 113–126.
2. Falcone T, Attaran M, Bedaiwy MA, Goldberg JM. Ovarian function preservation in the cancer patient. *Fertility and Sterility*. February 2004;81(2):243-57.
3. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. *Fertility and Sterility* February 2010;93(3):762-8.
4. Kim SS. Fertility preservation in female cancer patients: current developments and future directions. *Fertility and Sterility*. January 2006;85(1):1-11.
5. Grynberg M, Poulain M, Sebag-Peyrelevade S, Parco SI, Fanchin R, Frydman N. Ovarian tissue and follicle transplantation as an option for fertility preservation. *Fertility and Sterility*. June 2012;97(6):1260-8.
6. Fatemi HM, Kyrou D, Al-Azemi M, Stoop D, Sutter PD, Bourgain C, et al. Ex-vivo oocyte retrieval for fertility preservation. *Fertility and Sterility*. April 2011;95(5):1787.15-7.
7. Greve T, Schmidt KT, Kristensen SG, Ernst E, Andersen CY. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. *Fertility and Sterility* June 2012;97(6):1394-8.

8. Kim SS, Battaglia DE, Soules MR. The future of human ovarian cryopreservation and transplantation: fertility and beyond. *Fertility and Sterility*. June 2001;75(6).
9. Jain JK, Paulson RJ. Oocyte cryopreservation. *Fertility and Sterility*. October 2006;86(3):1037-46.
10. Oktay K, Karlikaya GG, Aydin BA. Ovarian cryopreservation and transplantation: basic aspects. *Molecular and Cellular Endocrinology* 169 (2000) 105–108. 2000.
11. Revel A, Revel-Vilk S, Aizenman E, Porat-Katz A, Safran A, Ben-meir A, et al. At what age can human oocytes be obtained? *Fertility and Sterility*. August 2009;92(2):458-63.
12. Ultrastructure of follicles after vitrification of mouse ovarian tissue. *Fertility and Sterility*. September 2002;78(3).
13. Amorim CA, Dolmans M-M, David A, Jaeger J, Vanacker J, Camboni A, et al. Vitrification and xenografting of human ovarian tissue. *Fertility and Sterility*. 2012; *article in press*.
14. Nottola SA, Camboni A, Langendonck AV, Demylle D, Macchiarelli G, Dolmans M-M, et al. Cryopreservation and xenotransplantation of human ovarian tissue: an ultrastructural study. *Fertility and Sterility*. July 2008;90(1):23-32.
15. Oktay K, Turkoglu I, Rodriguez-Wallberg KA. Four spontaneous pregnancies and three live births following subcutaneous transplantation of frozen banked ovarian tissue: What is the explanation? *Fertility and Sterility*. February 2011;95(2):804.7-10.
16. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. *Fertility and Sterility*. 2003;80:193-8.
17. Donnez J, Squifflet J, Dolmans M-M, Martinez-Madrid B, Jadoul P, Langendonck AV. Orthotopic transplantation of fresh ovarian cortex: a report of two cases. *Fertility and Sterility*. October 2005;84(4):1018.1-3.
18. Akar ME, Carrillo AJ, Jennell JL, Yalcinkaya TM. Robotic-assisted laparoscopic ovarian tissue transplantation. *Fertility and Sterility*. March 2011 95(3).
19. Kim SS, Lee WS, Chung MK, Lee HC, Lee HH, Hill D. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. *Fertility and Sterility*. June 2009;91(6):2349-54.
20. Kolp LA, Hubayter Z. Autotransplantation of cryopreserved ovarian tissue: a procedure with promise, risks, and a need for a registry. *Fertility and Sterility*. May 2011;96(6):1878-86.
21. Oktay K, Aydin BA, Karlikaya G. A technique for laparoscopic transplantation of frozen-banked ovarian tissue. *Fertility and Sterility*. June 2001;75(6):1212-6.
22. Dittrich R, Laura Lotz, Keck G, Hoffmann I, Mueller A, Beckmann MW, et al. Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation. *Fertility and Sterility*. February 2012;97(2):387-90.
23. Ploteau S, Rogez J-M, Donnez J, Lengele B. Which are the ideal donor and recipient vessels for a whole ovarian transplantation? *Fertility and Sterility* February 2011;95(2):751-5.
24. Bedaiwy MA, Shahin AY, Falcone T. Reproductive organ transplantation: advances and controversies. *Fertility and Sterility*. December 2008;90(6):2031-55.
25. Oktay K, Buyuk E. Ovarian transplantation in humans: indications, techniques and the risk of reseeded cancer. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2004;113S:S45-7.