Ovarian Cryopreservation and Transplantation: A Preserving Fertility Procedure

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Abstract

Developments in cancer treatment modalities and the ability to detect it in the early stages have increased survival rate, yet raise fertility problems for those who have to endure gonadotoxic therapy. Cryopreservation of either ovary, oocytes or embryo can be an option, surely with its own advantages or disadvantages. Cryopreservation procedures are not without risks. As an example, there is a risk of reimplanting an occult tumour within the frozen–thawed ovarian pieces. Therefore, a thorough discussion between the practitioner and patient regarding all available options should be performed prior to its implementation, and there should be a clear understanding that most fertility preservation options are currently experimental.
Keywords*: cryopreservation, fertility, cancer*

Kriopreservasi dan Transplantasi Ovarium: Prosedur Preservasi Fertilitas

Abstrak

Perkembangan modalitas tata laksana kanker dan kemampuan untuk mendeteksinya pada stadium awal dapat meningkatkan *angka kelangsungan hidup*, tetapi dapat meningkatkan masalah fertilitas pasien yang memerlukan terapi gonadotoksik. Kriopreservasi baik ovarium, oosit atau embrio dapat menjadi pilihan dengan keuntungan dan kerugian masing-masing. Prosedur kriopreservasi bukannya tanpa risiko. Sebagai contoh, terdapat risiko implantasi tumor di bagian ovarium yang dibekukan. Oleh karena itu, penggunaannya harus didiskusikan antara pasien dan dokter mengenai pilihan yang tersedia dan penjelasan bahwa pilihan preservasi kesuburan saat ini masih bersifat eksperimental.
Kata kunci: kriopreservasi, kesuburan, kanker

Introduction

Cancer is a major health problem in both developed and developing countries.[1] In women, cancer incidence rates increase every year. Developments in treatment modalities and the ability to detect tumours in the early stages increase survival rate, however, they raise fertility problems as well. Patients who utilized those modalities, such as: gonadotoxic chemotherapy and/or radiation have risks of developing fertility problems, even though they still need their fertility functions. Many treatments that administered for pediatric and adolescent cancer patients carry a substantial risk for infertility.[2] This risk varies according to the presenting pathology and requires preventive treatment. It was estimated that 1 in 1,000 adults was a survivor of childhood cancer.

Over the last decade, the field of ovarian transplantation and cryopre-servation significantly progressed, becoming applicable to humans. Cryopreservation of embryos is a standard technique for fertility preservation when there is adequate time for ovarian stimulation.[1] However, this technique requires at least 2 weeks from the beginning of the menstrual cycle, which may not be applicable to all patients with cancer. Furthermore, embryo cryo-preservation requires a partner and ovarian stimulation, both of which are not possible in prepubertal girls.[3] Ovarian cryopreservation and auto transplantation are another methods and initially designed to protect and restore reproductive function in patients receiving sterilizing chemotherapy and/or radiotherapy. Other indications: patients undergoing haematopietic 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 chemo-therapeutic drugs. Currently, the indications 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 indicated.[2] Previously, 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, or even 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 diagno-sed in women who are under the age of 50. With the typical regimen of cyclophosphamide, methotrexate, fluorouracil (CMF), two-thirds of women will become amenorrhea. With the doxorubicin, cyclophos-phamide (AC) protocol, 34% will be amenorrheic in 3 years. This per-centage increases if taxanes are also used with this regimen.

Indication for Ovarian Tissue Cryoperservation[1]

1. Cancer in children
2. Breast cancer
3. Cervical cancer
4. Autoimmune and hematological disease
5. Surgery for benign ovarian disease
6. Patients receiving pelvic radiation
7. Prohylactic oophorectomy

Ovarian Cryopreservation

Cryopreservation of ovarian cortex combined with orthotopic transplantation into an irradiated ovary restored fertility in rodents 50 years ago.[5] 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 fertility.[6-7] A major advantage of ovarian cryopreservation is the technique does not delay cancer treatment, unlike cryopreservation of embryos/oocytes.[8] Cryopreservation of oocytes and gonadal tissues is a rapidly evolving area in reproductive medicine.[9] It relies on the principle that the primordial follicles withstand the cryotoxicity better than the growing follicle. A relatively inactive metabolism, lack of zona pellucida, and metaphase spindle are the privileges owned by the primordial cells. Smaller cell size allows faster penetration of cryoprotectants in the oocyte.[10,11]The main factor that may influence the outcome in oocyte cryopreservation is its structural complexity. Oocyte subcellular organelles are more complex and possibly more sensitive to thermal injury than preimplantation embryos

Xenografting Studies of Human Ovarian Tissue

Cortical fragments of human ovarian tissue can be xenotrans-planted into a T- and B-cell deficient SCID mice. It was demonstrated that cryopreservation and xeno transplantation did not appear to greatly affect the human primordial/ primary follicle ultrastructure. Interestingly, in frozen

thawed xenografts, secondary human ova-rian follicles presented a well preserved ultrastructure, however, 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, GnRHa caused an additional loss of follicles if administered during the critical neovascularisation period after the transplantation. Until today, its use as a mean to use banked ovarian tissue is still 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, as 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]

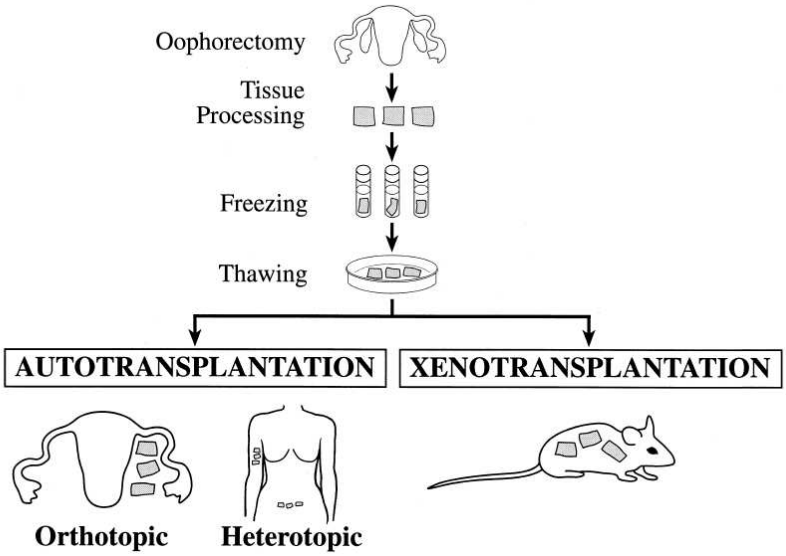

Fig 1. Options for Development of Immature Oocytes in Cryopreserved Ovarian Tissues[4]

It is still unknown, however, whether conditions for the growth and maturation of human oocytes in an animal host are comparable to those in situ. It is also a concern that animal pathogens can be transmitted to human tissue with xenografting.[8,14-15]

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.[16] In 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 there is a high risk of metastasis, considering the difficulty in tissue monitoring.[17]

Heterotopic Transplantation

In this technique, general anaesthesia or abdominal surgery is not required, follicle monitoring and, when necessary, removal of transplanted tissue are easier to be conducted in the subcutaneous site. Various body sites can be used to graft ovarian pieces, such as: subcutaneous space above the brachioradialis facia of the forearm or under rectus sheet 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, the heterotopic transplantation gives benefit in uncomplicated tissue monitoring.

There are numerous challenges in 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 the 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. Nonetheless, 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 mm^3 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, transmitted electron microscopy data suggest that vitrification could be more effective than slow programmed freezing when cryopreserving the ovarian tissue.

Whole Ovary Cryopreservation

Cryopreservation of a 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 cryopre-servation 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 has reported on the functionality of ovaries frozen intact.[23]

The Danger

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 purpose of future auto transplantation 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.[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 metastasis.[25]

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

Conclusion

Ovarian tissue cryopreservation is an option for fertility preservation for prepubertal girls and for women who face the high likelihood of diminished ovarian reserve due to requiring immediate cancer treatment. The procedure is still within improvement and also is still being studied for 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.

References

1. Sonmezer M, Oktay K. Ortho-topic and heterotopic ovarian tissue transplantation. Best Practice & Research Clinical Obstetrics and Gynaecology. 2010;24:113–26.
2. Falcone T, Attaran M, Bedaiwy MA, Goldberg JM. Ovarian function preservation in the cancer patient. Fertil Steril. 2004;81(2):243-57.

3. Oktay K, Oktem O. Ovarian cryopreservation and transplan-tation for fertility preservation for medical indications: report of an ongoing experience. Fertil Steril. 2010;93(3):762-8.
4. Kim SS. Fertility preservation in female cancer: current develop-ments and future directions. Fertil Steril. 2006;85(1):1-11.
5. Grynberg M, Poulain M, Sebag-Peyrelevade S, Parco Sl, Fanchin R, Frydman N. Ovarian tissue and follicle transplantation as an option for fertility preservation. Fertil Steril. 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. Fertil Steril. 2011;95(5):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. Fertil Steril. 2012;97(6): 1394-8.
8. Kim SS, Battaglia DE, Soules MR. The future of human ovarian cryopreservation and transplan-tation: fertility and beyond. Fertil Steril. 2001;75(6).
9. Jain JK, Paulson RJ. Oocyte cryopreservation. Fertil Steril. 2006;86(3):1037-46.
10. Oktay K, Karlikaya GG, Aydin BA. Ovarian cryopreservation and transplantation: basic aspects. Molecular and Cellular Endocrino logy. 2000;169:105-8.
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? Fertil Steril. 2009; 92(2):458-63.
12. Ultrastructure of follicles after vitrification of mouse ovarian tissue. Fertil Steril. 2002;78(3).
13. Amorim CA, Dolmans M-M, David A, Jaeger J, Vanacker J, Camboni A, et al. Vitrification and xeno grafting of human ovarian tissue. Fertil Steril. 2012; in press.
14. Nottola SA, Camboni A, Langendonckt AV, Demylle D, Macchiarelli G, Dolmans M-M, et al. Cryopreservation and xenotrans plantation of human ovarian tissue: an ultrastructural study. Fertil Steril. 2008;90(1):23-32.
15. Oktay K, Turkoglu I, Rodriguez-Wallberg KA. Four spontaneous pregnancies and three live births following subcutaneous transplant ation of frozen banked ovarian tissue: What is the explanation? Fertil Steril. 2011;95(2): 8047-10.
16. Oktay K, Buyuk E, Rosenwaks Z, Rucinski J. A technique for transplantation of ovarian cortical strips to the forearm. Fertil Steril. 2003;80:193-8.
17. Donnez J, Squifflet J, Dolmans M-M, Martinez-Madrid B, Jadoul P, Langendonckt AV. Orthotopic transplantation of fresh ovarian cortex:a report of two cases. Fertil Steril. 2005;84(4):1011-3.
18. Akar ME, Carrillo AJ, Jennell JL, Yalcinkaya TM. Robotic-assisted laparoscopic ovarian tissue trans plantation. Fertil Steril. 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. Fertil Steril. 2009;91(6):2349-54.
20. Kolp LA, Hubayter Z. Autotransplantation of cryopre-served ovarian tissue: a procedure with promise, risks, and a need for a registry. Fertil Steril. 2011;96(6):1878-86.
21. Oktay K, Aydin BA, Karlikaya G. A technique for laparoscopic trans-plantation of frozen-banked ovarian tissue. Fertil Steril. 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. Fertil Steril. 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? Fertil Steril. 2011;95(2): 751-5.
24. Bedaiwy MA, Shahin AY, Falcone T. Reproductive organ transplanta tion: advances and controversies. Fertil Steril. 2008;90(6): 2031-55.
25. Oktay K, Buyuk E. Ovarian transplantation in humans: indica-tions, techniques and the risk of reseeding cancer. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2004; 113S:S45-7.