Original Research Article

Ovarian tissue cryopreservation as an option for fertility preservation in young unmarried girls suffering from cancer

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Abstract

In the present society, women in the reproductive age group delay their marriages, due to various social and economic reasons and the age of bearing their first child also gets delayed. It is well known fact that women steadily lose their oocytes from birth to menopause. Moreover, if the woman develops some kind of malignancy in due course, it leads to infertility due to unwanted gonadotoxic side effects of cytotoxic chemotherapeutic drugs and/or radiotherapy. Although survival in case of malignancy takes utmost priority, infertility also is an issue of prime concern which haunts such patients. In such patients ovarian tissue cryopreservation remains one of the methods of fertility preservation. This method has the advantage of requiring neither the sperm donor nor the ovarian stimulation as both these requirements presents ethical and social issues for an unmarried girl. In young unmarried girls suffering from malignancy, where IVF-ET (In Vitro-Fertilization-Embryo Transfer) is contraindicated, ovarian tissue cryopreservation and transplantation could become the technique of choice in the future.

Keywords: Ovarian cortex, cryopreservation, fertility preservation, cryoprotectant

Introduction

The first attempts of cryopreservation of ovarian tissue were reported in the year 1987[1]. Since then a number of cryobiologists have reported live births from frozen-thawed ovarian cortical tissue[2, 3]. Controlled rate freezing was initially used as a method of cryopreservation of ovarian tissue[4, 5]. Vitrification of ovarian tissue was initially successful in the animal models and proved to be an effective and alternate method of cryopreservation of human ovarian cortex[6, 7]. Vitrification of ovarian tissue was widely studied in sheep. Retrieval of oocytes followed by their in vitro maturation to metaphase II stage from the vitrified ovarian tissue was successfully carried out[8]. In the subsequent years, attempts were made to vitrify whole sheep ovaries by perfusion of vitrification solution in their ovarian vessels[9]. Bordes et al. reported the birth of four lambs after auto transplantation of vitrified warmed ovarian cortex into ewes[10]. Ovarian cortex freezing in young girls undergoing chemotherapy and/or radiotherapy has become an established as a method of fertility preservation[11, 12]. The reporting of live births from frozen-thawed auto transplanted tissue has further established this method in women who are at risk of undergoing premature ovarian failure as a result of chemotherapy and radiotherapy[13, 14]. A significant increase in premature ovarian failure (POF) has been observed in cancer survivors[15]. This novel procedure has ethical and moral issues which need to be addressed[16]. The decision to operate on a young patient for obtaining the ovarian tissue needs justification especially when there is limited success of the whole procedure[17].

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Our Experience

Cryobiology unit of our Assisted Reproductive Technology Centre carried out cryopreservation of ovarian cortical tissue from 8 patients since Jan 2009. All these patients were referred from the oncology department for fertility preservation prior to chemotherapy and /or radiotherapy. The patients were carefullyselected following oncological and gynecological assessment.This method has been applied to young unmarried girls and adult married females. Our series included female patients 13-36years old. Indications for ovarian banking in these patients included,carcinomas of genital tract, lymphomas, leukemia and patients prior to(about to undergo) Bone Marrow Transplantation.

Method of Ovarian cortex freezing

Time and experience has proved vitrification to be better modality of ovarian tissue cryopreservation however we used slow freezing in maximum of our patients as it was the best established and available modality at that time.

Whole ovary or part of ovarian tissue was removed under laparoscopy under general anesthesia and transferred immediately to warm (37^0C) HEPES-buffered flushing medium. A small sample (fresh cortex) was removed, for routine histopathology and follicle density, prior to cryopreservation. The fragments are placed in a pre-cooled standard IVF medium and kept on ice for transportation to embryology laboratory.

In the laboratory the procedure is carried out on a workstation under the laminar flow. The plastic container containing the ovarian tissue was placed in an icebox. The whitish cortex is separated from the reddish appearing medullary tissue using a tissue cutting scissors, surgical blade and toothed forceps. The cortex is sliced to 1-2 mm of thickness and approx.1cm of length and these slices are rinsed several times with cryoprotectant solution.

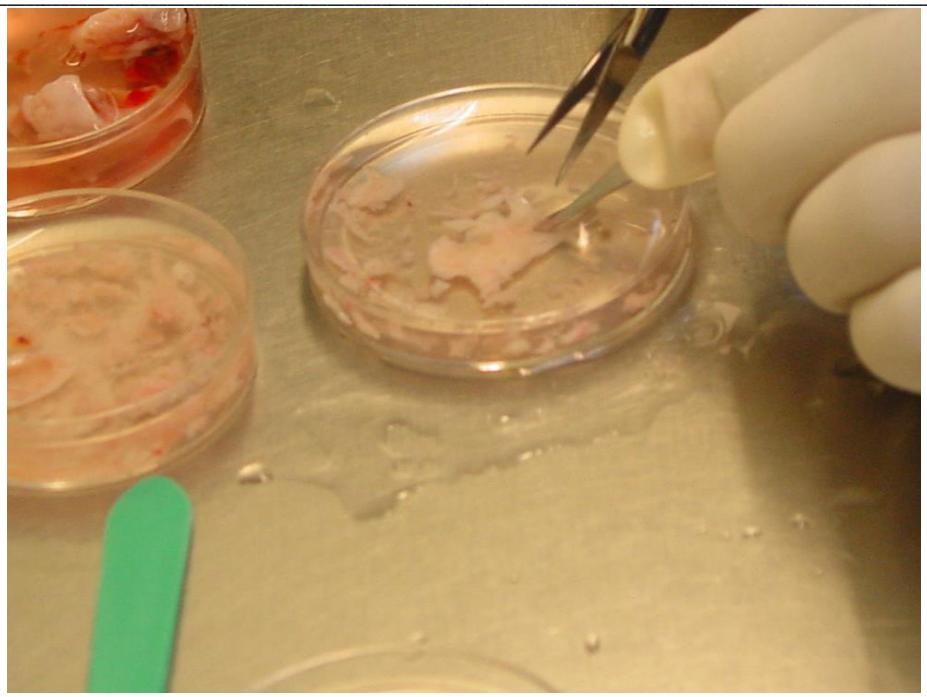

Fig.1 Ovarian cortical tissue strips prepared for cryopreservation

All the slices of tissue are placed on the ice cooled cryoprotectant solution in a 90mm petridish (Falcon,USA). 5-6 cryovials (Nunc A/S, Denmark) are labeled and the tissue slices are transferred to these cryovials using a plain forceps. The fragments of cortex are stored in 1.8 ml cryovials, each containing 1 ml of cryoprotectant, and cryopreserved using an ovarian tissue freezing programme (Freezol, France).

Fig.2 Cryopreservation equipment (Freezal, France)

The following programme is used: 2°C/min to -9°C, 5 min of soaking, then manual seeding for ice crystal nucleation induction, 0.3°C/min to -40°C, 10°C/min to -140°C, at which temperature the samples were plunged into liquid nitrogen at -196°C. After the freezing programme is over, the cryovials are placed on the pre-labelledpre-cooled cryocanes for storage in liquid nitrogen.

Fig.3 Liquid nitrogen cryocans used for storage of cryopreserved ovarian tissue

The permeating cryoprotectant such as Dimethylsulfoxide (DMSO) or ethylene glycol is used in combination with a non-permeating cryoprotectant such as sucrose. For successful cryopreservation, the cryoprotectant agent should uniformly penetrate the ovarian cortical tissue.

This cryopreserved ovarian cortex tissue is intended to be thawed and implanted after completion of chemotherapy and/or radiotherapy. Once the patient is cured of its malignancy, the thawed ovarian cortical tissue can be transplanted in cases of treatment induced ovarian failure[9, 10]. Ovarian tissue can be transplanted orthotopically to pelvis or heterotopically to forearm or lower abdomen subcutaneously[11, 12]. Although this procedure was introduced more than 14 years ago, experience with auto transplantation of frozen-thawed ovarian tissue is still in the experimental stage and only a few cases have been successful[13]. Vitrification has proven to be technique of choice of current times. It's technically easier than slow freezing and gives better results. The technique of vitrification involves direct exposure of cryoprotectant treated ovarian. cortex tissue strips to liquid nitrogen. This process avoids damage caused by ice crystal formation and osmotic stress caused by solute distribution in slow freezing

Discussion

The main aim is the cryopreservation of follicles within strips of ovarian cortical tissue. The integrity and viability of the tissue after cryopreservation depends on the condition of non-frozen tissue used. There remains a large variation in morphology of the tissue from different patients and from different fields of the ovary. When the ovarian cortical tissue sections were cryopreserved using slow freezing procedures it led to the development of the ice crystals which proved detrimental to the cells. Hence, vitrification of the ovarian tissue became an established method for preservation with no ice crystal formation[18]. The vitrifying solution remains unchanged and is cooled into a glass like state due to increase in the viscosity of the solution during cooling[19]. Different variables affect the efficiency of ovarian tissue cryopreservation which includes type of the cryoprotectant, cooling rate, type of procedure employed and the size of the ovarian tissue sections taken.Vitrification results in maintaining good morphology because formation of ice crystalsin the cryopreserved sample can be avoided.The concentration of each cryoprotectant is increased in astepwise manner.

Majority of the follicles in the cryopreserved ovarian cortical tissuecannot survive the cryoinjury except the primordial follicles which show a survival rate of 70% after thawing[20]. Development of the immature oocytes in a culture system followed by the transfer of embryos to the patient after chemotherapy and/or radiotherapy remains an important strategy[21]. Autotransplantation of the ovarian cortical tissue is a good strategy but has the risk of transmitting the malignant cells in metastatic disease[22]. Numerous animal studies have shown that transplantation of ovarian cortex tissue from animals and humans, to immunodeficient mice, supports the development of follicles[23, 24, 25]. The study of Kim et al showed the evidence of ovulation and corpus luteum formation, after hCG injection in human ovarian cortical tissue xenografted to immunodeficient mice[26]. The most important factor for survival of the graft is the revascularization of the follicles. Ischemia is the main cause of the follicular loss after transplantation of the ovarian cortical tissue. Hence the subcutaneous space has emerged as the preferred site for the transplantation because it has easy access, provides ample space for follicular development and also has the ease of follicular monitoring by ultrasound. But the vascularity of subcutaneous space is less as compared to other sites like the subcapsular spaces of the kidney. The development of follicles in animal hosts is compromised after xenotransplantation[27]. The maturity of the oocytes in the ovarian cortex transplanted to a heterotopic site was significantly less as compared to orthotopic sites[28]. Much information has been gained about the nuclear maturity of the human oocytes in the antral follicles grown in the xenografts[29]. About 20 weeks are required for the development of the first pre-ovulatory follicle which is independent of age of patient, amount of tissue transplanted and the number of follicles present in the transplant[30]. Some of the follicles survive the cryopreservation and resume their growth after transplantation as seen in morphologically normal follicles in frozen thawed ovarian tissue[31]. Auto transplantation of frozen thawed ovarian cortical tissue can restore ovarian function with a fair degree of efficacy and the life span of the grafted tissue is enough to buy sufficient time for assisted reproduction for conceiving.Moreover, the success of the ovarian tissue graft may be influenced by many factors including

follicular density and follicular volume[32]. It is recommended to do ovarian tissue banking for young women with cancer as they might lose their chances of fertility and subsequently the joy of motherhood after cancer treatment. Also, the ovarian tissue banking will not delay the anticancer treatment significantly.

Conclusion

In order to offer the best options for fertility preservation, oncologists should work in close collaboration with the infertility specialists, embryologists and psychologists. It assumes importance especially for the young girls with malignancy, who are undergoing gonadotoxic chemotherapy and /or radiotherapy. Furthermore, other methods of fertility preservation should be explored in cancer patients in order to give them hope for the future to meet their dream of parenthood.

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