Recent Advances in Endometrial Cancer

Avid Science
Chapter 1

Cryopreservation of Ovarian Tissue in Endometrial Cancer Patients

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Abstract

The annual incidence rate of cancer is estimated more than 11,000 patients in UK in the age group of 15-40 years old, which corresponds to 4% of all cancer patients. The diagnosis of cancer is followed by devastating consequences for the patients themselves and their families in this age group. Although the treatment of cancer is of crucial significance, it should also examine the impact of the disease on fertility at the time of diagnosis and the damages caused from the surgical treatment, chemotherapy or radiotherapy [1].

The gynecological cancer, the prevention and treatment, as well as the prevention of fertility in young women who are affected with the consequences of cancer remain the gold standard of the gynecologists. Infertility, whether temporary or permanent and premature menopause are common complications of the therapeutic approaches of cancer. The anticipated increase of the incidence of gynecological cancer in ever younger patients has led the medical community to specific efforts towards the prevention of fertility both through surgical practice and also through cryobiology, this scientific field, which studies the effect of low temperatures on living organisms. Namely cryobiology in terms of assisted reproduction, enables the process of cryopreservation of gametes, embryos and ovarian tissue.

Keywords

Cryopreservation; Endometrial Cancer; Ovarian tissue
Introduction

According to World Health Organization, infertility is defined as failure to conceive after free intercourse for at least one year. The American Society of Reproductive Medicine recommends that women over 35 years should consult a specialist gynecologist after free intercourse lasting six months, while in women aged over 40 after three months. In 10% of couples, the infertility factors refer to both the man and the woman. 10% idiopathic infertility remains even after laboratory and imaging evaluation [2].

Infertility is a global health issue that affects a percentage of 8-10% of couples. This is a multifaceted problem with social, economic and cultural consequences, which can take threatening proportions in countries with demographic problems, such as Greece. In recent times an increasing number of couples with infertility problems opt for assisted reproduction techniques [3].

The causes of infertility comprise the male factor 25-40%, the ovulation disorders 10-15%, the peritoneal tubal factor and the endometriosis 30-40%, factors related to the uterus 10-15%, the cervical factor 10-15% and the idiopathic infertility 10-15% [4].

The European Society for Human Reproduction and Embryology (ESHRE) emphasizes that the prevalence of infertility is increasing in the developed world. The main reasons are the tendency for future postponement of pregnancy, the increase in obesity and the highest rate of sexually transmitted diseases. Doctors state that these changes in health and community level could lead to a rapid increase fertility treatment demand in the coming years. Fertility treatments can be grouped into three categories: medication in improving fertility, surgery and assisted reproductive techniques [5].

The latest 30 years has been made considerable progress in the area of assisted reproduction, either through development of new techniques or the progressing pharmacology. In 1978 after a long and strenuous research effort of Robert Edwards and Patrick Steptoe performed the birth of the first child of Louise Brown, through embryo transfer.

Brief History of IVF

1961: Description of first egg retrieval via laparoscopy by Palmer.

1965: Robert Edwards tried to fertilize human oocytes under laboratory.

1973: It takes place in Australia the first IVF which ended in miscarriage.

1976: Steptoe and Edwards described the first ectopic pregnancy from IVF.

1978: July 28th, the first birth mentioned in England on IVF from the above doctors.

1983: Performed the first pregnancy from oocyte do-
nation and then the first childbirth.

1984: Introduced in Victoria the first law on IVF. Also reported the first birth in Greece with IVF.

1990: First successful cryopreservation of human embryos by vitrification method.


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2000:

- First mention transplant ovarian tissue of Oktay and Karlikaya.

Figure 1: Stages of fertilization and implantation of the embryo.

Methods of Preservation of Fertility in Women with Cancer

Standard Methods

IVF: In vitro fertilization is a widely used technique worldwide.

Cryopreservation of embryos (reception of oocytes, fertilization in the laboratory and freeze embryos). The success rate is slightly lower compared to the implantation of fresh embryos.

Borrowing of ova and uterus lending: Indications for egg donation are premature ovarian failure, menopause (automatically or postoperative), the chromosomal abnormalities, prior chemotherapy or radiotherapy, poor repeated attempt IVF caused of oopenias, familial disease which is not easy to diagnose. The indications for uterus lending are the absence of the uterus or hysterectomy syndrome Rokitansky-Kuster-Hauser, anatomical malformations on the conformation of the uterus, severe organic disease such as hypertension or diabetes [6,7].

Displacement ovaries laparoscopically before radiotherapy in the pelvic cavity (height renal vessels) that is not to be irradiated [8].

New Techniques

- Cryopreservation of unfertilized ovums.
- Receive of immature oocytes matured in the laboratory, ovum freezing, embryo freezing.

Techniques Ongoing

- Cryopreservation of ovarian tissue and autotransplantation: Freezing ovarian tissue regards tissue, which obtained prior to chemotherapy through laparoscopy and
then undergoes maturation of ova in laboratory conditions [9].

**Figure 2:** Schematic representation of ovarian tissue reception.

**Suppression of ovarian** through hormone therapy during chemotherapy or radiotherapy [10,11].

As apparent word cryopreservation is derived from the word cold term denoting freezing cells or tissue, usually in liquid nitrogen at temperatures -130°C. The main goal of cryopreservation process is the minimization of tissue injury of the low sub-zero temperatures. Maintenance at low temperature can go on for decades. The lowest natural temperature on Earth is -80 °C. Under normal pressure the inert nitrogen gas which is commonly used in cryopreservation becomes liquid at -196 °C [12].

Cryopreservation is based on a process of cooling and preservation of samples in liquid nitrogen at temperatures -196°C. Separated into two main methods: the method of slow freezing (slow freezing) and vitrification (vitrification) [13].

**Figure 3:** Fertility preservation Pyramid [14].

The cryopreservation of ovarian tissue can be considered the most suitable method for pre-adolescent patients undergoing chemotherapy, pelvic radiation therapy, haematopoietic stem cells or ovariectomy on benign diseases.
Also problems of oocyte cryopreservation as the time required and the ethical issues, suspended from the cryopreservation of ovarian tissue [15].

The harvest of the ovarian tissue can be easily accomplished by laparoscopy. The ovarian cortex resected into small pieces after purified from bone, can diffuse more readily the cryoprotective agent in the cortical tissue [15].

The tissue is removed by laparoscopy or laparotomy procedures. The bark is removed carefully from the layer, cut into strips and frozen by slow freezing protocol using dimethylsulfoxide (DMSO) as cryoprotectant. 5% of fresh tissue is cultured with a mixture of collagenase, pronase and DNAses at 37 °C for 60 minutes to arise follicles. The survival of the follicles and ova studied using the LIVE / DEAD Viability / Cytotoxicity Assay Kit under fluorescent microscope. The procedure was repeated with 5% frozen tissue of the same patient after thawing, to evaluate the potential tissue damage due to freezing and thawing. The study of survival granulocytes and oocytes will enable us to improve the download, freezing and thawing ovarian tissue and thus increase the effectiveness of the method.

Figure 4: Ovarian tissue removable laparoscopically [15].

Figure 5: Pieces of ovarian cortex prepared for cryopreservation [15].

Receive, freezing and thawing ovarian tissue is used
effectively in the past decade. By freezing a plurality of stored primary follicles containing small oocytes undifferentiated. After the first birth published by Donnez 2004, freezing of ovarian tissue has already resulted in eight successful births after about forty retransplantation worldwide [17].

![Figure 6: Steps of ovarian tissue transplant procedure][16]

The cancer is rare in women of reproductive age. About 8% of cancer cases among women in the US are shown under the age of 40 years. During the last three decades the survival rate of young women undergoing chemotherapy due to cancer or autoimmune diseases has increased significantly due to the improved chemotherapeutic regimens [18].

![Figure 7: Strips of ovarian cortex.][16]

The slow-freezing protocol is the standard ovarian tissue freezing protocol. According to the protocol used by the Donnez, the vials are placed in the freezer (Kryo 10, Series III; Planer, Sunbury-on-Thames, UK) according to the following schedule: (1) cooling from 0 °C to -8 °C at a rate of -2 °C / min, (2) “seal” (seeding) vials by hand using pliers have cooled before in liquid nitrogen, (3) cooling at -40 °C at a rate -0 • 3 °C / min, (4) cooling to -196 °C at a rate of -30 °C / min and (5) direct placement into liquid nitrogen.
nitrogen (-196 °C) for storage.

The fluorescence microscopy to assess the vitality of the tissue takes place in Biomedical Research of the Academy of Athens Foundation then use the existing fluorescent microscope Olympus (Olympus BX60, Olympus USA). The kit LIVE / DEAD® Viability / Cytotoxicity Assay Kit with calcein AM and ethidium homodimer (EthD-1) used in the assessment of the loss of follicles. Following enzymatic digestion with a mixture of collagenase, pronase and DNA at 37 °C for 60 minutes for extraction of intact follicles, follicles are placed in a solution of 100 ML phosphate buffer (Dulbecco PBS, Sigma-Aldrich Co., Irvine, UK) which It contains 2 microns Calcein-AM and 5 microns Ethidium-Homodimer-I (LIVE / DEAD® Viability / Cytotoxicity Assay Kit, Invitrogen UK). The solution was incubated for 45 min at 37 °C in the dark. The calcein is absorbed by the live cells, producing an intense uniform green fluorescence (ex / em ~ 495 nm / ~ 515 nm). The EthD-1 enters cells with damaged membranes and undergoes an increase in fluorescence 40 fold by binding to the nucleic acids, producing a bright red fluorescence in dead cells (ex / em ~ 495 nm / ~ 635 nm), while not permeate intact cell membranes of live cells. Accordingly, the results are compared to assess necrosis and tissue damage.

**Discussion**

The ovarian tissue freezing and retransplantation thereof into the ovarian fossa is one of the most modern processes preserve fertility in women diagnosed with cancer, particularly young and undergoing radiation or chemotherapy. The majority of young people, diagnosed with cancer, are considered long-term survivors. Fertility preservation difficulty is real and can not be prevented. In many cases the research is growing and evolving in order to offer cancer patients the possibility of obtaining biological offspring in the future. Several scientific groups but also within society specific guidelines and legislation concerning the consent of fertility preservation have been developed. They have to take decisions about fertility and the imminent genotoxic therapies. The timely and full information about the effects of cancer treatment and fertility preservation options should be presented in all patients, especially when planned some kind of cancer treatment [19].

Recently, significant progress has been achieved in the field of cryopreservation of ovarian tissue, one of the only options for preserving fertility in women who require immediate gonadotoxic chemotherapy [20].

Cryopreservation of ovarian tissue has the objective of maintaining the oocytes within primordial follicle in the ovarian cortex. Receiving the ovarian tissue can be performed laparoscopically, programmable shortly after diagnosis of the malignancy and requires hormonal stimulation.
It is preferable to receive tissue for cryopreservation before initiation of genotoxic therapy. However it may be performed after the first dose of chemotherapy in young women, who often have large numbers of stem follicles in the ovaries. Repositioning ovarian tissue has been shown to provide recovery of ovarian function and spontaneously restores fertility. Hormonal stimulation with gonadotropins and success after IVF has been reported in women with ovarian tissue repositioning after cryopreservation. The ovarian tissue can be obtained orthotopically, i.e. the anatomic endopelvic ovarian skill Heterotopic, i.e. elsewhere, including outpelvic areas. The techniques used to reposition ovarian tissue currently being improved, in particular to reduce the initial ischemic loss follicle in repositioned tissue [19].

The problem of infertility after cancer treatment is of major concern. The cryopreservation of ovarian tissue carried out in 2005 after surgery and transfer of tissue in cooling mode. In 2011 was established the infant born after freezing process and repositioning of ovarian tissue. The first pregnancy and live childbirth occurred in Germany [21].

The first incident ovarian tissue cryopreservation and effective repositioning driven in pregnancy in Germany described by Muller A et al. Patient 25 initially underwent chemotherapy and radiation because mediastinal Hodgkin's lymphoma in 2003 and suffered a relapse two years later. The ovarian tissue was performed laparoscopi-

cally and cryopreservation this. Then the patient received high dose chemotherapy. He remained in remission for five years the period in which there was inability to conceive of the patient. The cryopreserved ovarian tissue was thawed and repositioned in the peritoneal laparoscopic follicle into the ovarian fossa the anatomical region of the right pelvic wall. Three months later, it occurred appearance of the first spontaneous menstruation. Six months after the repositioning, after two normal menstrual cycles, the administration of low-dose FSH therapy caused the emergence of a dominant follicle. Ovulation was induced by administration of hCG, in which case the patient is arrested normally. After an uncomplicated pregnancy, the patient gave birth to a healthy child via caesarean section on 10 October 2011. The histological ovarian tissue sample examination indicated that within the grafted tissue amount follicles at different stages of development, in contrast to the original ovarian tissue containing only structures without reproductive capacity [22].

Father of retransplantation of ovarian tissue in young patients considered the Jacques Donnez, doctor gynecologist from Belgium, born in 1947. In 2003, Donnez with his partners succeeded repositioning ovarian tissue in a patient with Hodgkin's lymphoma stage IV, where the cryopreservation of ovarian tissue was held in 1997. Later achieved a successfully pregnancy and birth of a healthy newborn.
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Protocol Regarding the Examination of Ovarian Tissue Viability

LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells from Invitrogen/ Molecular Probes (Cat. No. L-3224)

Ex/Em (nm) calcein 494/517 Green (Live)
ethidium homodimer-1 517/617 Red (Dead)

The reagents of the kit are stored in -80°C protected from light and humidity.

Protocol:
- Remove the reagents from the freezer and allow them to warm to room temperature.
- Place tissue samples in wells of a 24-well plate and wash in PBS 3x.
- During washing prepare the dye solution in PBS as follows:
  - Calcein stock C=4mM, use at 2µM (1:2000 dilution).
  - Ethidium homodimer-1 stock C=2mM, use at 4µM (1:500 dilution).
- Incubate each sample in 2ml volume, so add 1µl of calcein and 4µl of EthD-1 per sample. Vortex vigorously to ensure thorough mixing.
- *Note that aqueous solutions of calcein are susceptible to hydrolysis, so they should be used only on the same day.
- After last wash, add the dye solution in the sample. In parallel, incubate a sample with PBS alone without the dye, as a negative control for the autofluorescence of the tissue.
- Incubate for 30min in a 37°C incubator.
- At the end of the incubation, wash the tissue 3x with PBS.
- Place tissue on a slide with PBS and observe the tissue unfixed under a confocal microscope.

References


Conclusion

Infertility is one of the most serious problems nowadays. In particular young women, without any pregnancy, of childbearing age affected by neoplasia are facing a major problem of not achieving future pregnancy.

Confronted with this dilemma, the ovarian tissue freezing is invited to reply to this problem. Through this doctoral study is demonstrated the viability of follicles in the ovarian tissue after the surgery, chemotherapy, radiotherapy and hormonotherapy potential.

The ultimate objective remains the achievement of a future pregnancy and acquisition of live, healthy offspring.
Greek reality: legal issues. OA Woman’s Health. 2014.


13. AbdelHafez FF, Desai N, Abou-Setta AM, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. Reproductive Bio-


21. Isachenko V, Isachenko E, Keck G, Dittrich R,