First Ovarian Tissue Cryopreservation and Autotransplantation in Patients with Malignancies in Hungary – Report of the First Three Cases

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Abstract: Autotransplantation of cryopreserved ovarian tissue is one of the most advanced methods for fertility preservation of patients suffering from malignant diseases. Even though the method itself is still experimental, nearly a hundred live births have been documented worldwide, and its efficacy is comparable with the efficacy of any other assisted reproductive technology. Our prospective, non-randomized study was the first in Hungary that aimed to examine the safety and efficacy of fertility preservation based on ovarian tissue cryopreservation and autotransplantation. Patients were included only with stage I-III malignancy confirmed by histological diagnosis with a high risk for post-treatment infertility. 13 patients met the inclusion criteria and were enrolled in the study. After successful treatment and recovery, cryopreserved ovarian tissue was thawed and autotransplanted in three cases. The ultrathin slices of ovarian cortex were transplanted on the remaining ovaries with laparoscopic or mini-laparotomic intervention. Patients were discharged home after an uneventful postoperative period and are followed up currently. In summary, cryopreservation and autotransplantation of ovarian tissue is a safe technology for fertility preservation, which should be considered to offer and perform prior to gonadotoxic treatment, after individual evaluation of patients.

INTRODUCTION

Recent development of treatments for malignant diseases led to a dramatic increase in long-term survival. At the same time, there is an increased attention to the quality of life of the survivors, as patients with malignancies have to deal with numerous medical and social problems. One of the most common problems that affecting quality of life of young female patients is the issue of fertility. The most common malignant diseases in girls and young adult women are breast cancer, cervical cancer, various leukemias and lymphomas, and malignancies of the central nervous system. Because of high gonadotoxicity, many chemotherapeutic protocols and pelvic radiation can lead to premature ovarian failure and infertility following recovery from the malignant disease (1). In breast cancer, for example, the incidence of the chemotherapy-induced amenorrhea is approximately 53 - 89% (2).

Recently, many attempts have been made to develop appropriate fertility preservation methods (3). In general, fertility preservation means the preservation of gonadal cells or tissues retrieved prior to the initiation of gonadotoxic treatment. After recovery from the original illness, these cells or tissues can be reintroduced into the body and their function may restore the reproductive potential of the patients.

According to recent recommendations of the American Society of Clinical Oncology (ASCO), the most well-established and recommended option for fertility preservation in postpubertal girls and adult women is oocyte or embryo cryopreservation after controlled ovarian stimulation (4). Today, several flexible stimulation protocols are available, therefore it is not necessary to follow the natural menstrual cycle in all cases. However, these methods also take at least 12 to 14 days, hence initiation of cancer therapy must be delayed. Furthermore, the response of cancer patients to ovarian stimulation is still a matter of debate in the literature. Cancer patients often have lower anti-Müllerian hormone (AMH) level and lower antral follicle count compared to healthy women. If the number of obtained oocytes and embryos is sufficient, the quality of these raises further questions in patients with malignant diseases (5).

Ovarian tissue cryopreservation and further autotransplantation, a novel method of fertility preservation, has several benefits and its effectiveness has been proved by numerous recent studies. Moreover, this is the only available option for fertility preservation in young prepubertal girls (4). One of the key benefits of the technique is that it not only preserves reproductive functions, but endocrine function of the ovaries may also be restored. Consequently, the technique relieves symptoms of amenorrhea, menopause and premature ovarian failure. The real risk of this method may occur in case of potential involvement of ovaries in the malignant disease, as malignant cells can be reintroduced into the body during transplantation, resulting in recurrence of the original disease. Because of these reasons, safety of fertility preservation by ovarian tissue autotransplantation is crucial. Numerous in vitro studies have been conducted to assess the potential risks of the methodology. As a conclusion, freezing and autotransplantation of ovarian tissue is considered to be safe in early, non-disseminated malignancies. Leukemia is an exception, where ovarian tissue freezing is recommended only in patients in complete remission (6).

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While presumably there are thousands of already frozen ovarian tissues worldwide, only few studies have been published so far on successful transplantations and subsequent live births. According to the most recent data, cryopreserved ovarian tissues were transplanted in 360 cases (6). In 87% of the cases, the reason of fertility preservation was various types of malignancy, most commonly breast cancer and haematological cancer. Relapses occurred in 9 of the 230 cases after ovarian tissue transplantation, but none of these was attributed to malignant contamination of the ovarian tissue. According to a comprehensive meta-analysis, endocrine function was restored in 85.2% of the patients after ovarian tissue transplantation, and the method resulted in live birth or clinical pregnancy in 37.7% of the cases (7). Another prospective cohort study examined 545 cases of ovarian tissue freezing and found 33% live birth rate, as 7 of the 21 transplanted patients gave birth to healthy children (8). Examining the perinatal conditions of children born using this method, it can be concluded that the technique works successfully, and its outcome is comparable to that of normal pregnancies (9). Although this method is still considered experimental in most countries, many experts recommend its reclassification to routine procedure in those cases where the risk of ovarian metastasis is low (10).

Other fertility preservation options include the application of gonadotropin releasing hormone (GnRH) analogues which could provide potential protection for the ovaries, and the surgical transposition of the ovaries prior to pelvic radiation therapy. However, the effectiveness of these methods is controversial and they can only be applied in certain cases, hence the ASCO guideline only recommends these if other methods, such as oocyte, embryo or ovarian tissue cryopreservation are not feasible (4).

In our current study, we aim to describe three cases of ovarian tissue freezing for fertility preservation and orthotropic autotransplantation of the tissue fragments after complete recovery of the patients. The interventions were performed at the Versys Clinics – Human Reproduction Institute in Budapest, Hungary. Our institute has been conducting ovarian tissue cryopreservation for fertility preservation since 2014 and these three cases were the first autotransplantations performed in Hungary.

MATERIALS AND METHODS

Study

This prospective, non-randomized study has started in December 2013 (permission number V-R-021/02529-11/2013). According to the general inclusion criteria of the study, patients with stage I-III malignancies indicated for gonadotoxic therapy were recruited. Only women under 38 years with normal ovarian functions and with ECOG performance status 0 were included. For the inclusion, patients needed to sign the informed consent form, and the Oncological Committee at our clinic and at the clinic responsible for the treatment of the patients must have given approval as well. Exclusion criteria were as follows: 1.) primary ovarian cancers, ovarian metastasis, or gynecological cancers which make the procedure impossible 2.) age over 38 3.) ECOG performance status › 0 4.) known severe infectious disease (HIV, hepatitis, etc) 5.) early menopause or insufficient ovarian functions 6.) IV. stage malignant disease, or malignancies with metastasis. After the diagnosis of the malignant disease and prescription of the gonadotoxic treatment, all patients signed an informed consent form. The patient's oncologist and the oncology committee of our clinic also gave their consent to the intervention both before tissue removal and before tissue autotransplantation.

Ovarian Tissue Removal – Surgical Procedure

Prior to cryopreservation, ovarian tissue sections were removed by laparoscopy. The adnexa were grasped with forceps and 25-30% of the ovarium on both sides were resected with sharp dissection using scissors. After the ovarian tissue sample had been removed, bleeding site of the ovarium was treated by bipolar electrocoagulation. The excised ovarian tissue was transferred to the freezing laboratory for further processing.

Ovarian Tissue Freezing

Ovarian tissue cryopreservation was performed according to Donnez et al. (15). The surgically excised ovarian tissue sections were placed in pre-cooled L-15 medium (Leibovitz with L-glutamine; Sigma-Aldrich) and the preparation was done at 4°C. The ovarian medulla was dissected from the cortex and the cortex was cut into 5x10 mm strips. One piece from both ovaries were placed in formalin for additional histological analysis. The remaining cortical strips were washed twice with L-15 medium and once with freezing medium (L-15 medium supplemented with 10% DMSO (Amresco) and 0.4% HSA (Vitrolife)). The tissue fragments were placed in cryovials containing freezing medium and they were frozen by slow freezing using a programmable freezer (Freeze Control CryoPreservation System; CryoLogic) as follows: 15 min incubation at 0°C, freezing to -8°C at a freezing rate of -2°C per min, manual seeding for ice crystal induction, 15 min incubation at -8°C, freezing to -40°C at a freezing rate of -0.3°C per min and to -150°C at a freezing rate of -30°C per min. The vials were placed in liquid nitrogen and stored at -196°C until use.

Ovarian Tissue Thawing

Once removed from liquid nitrogen, the cryovials were incubated for 2 min at RT and then for 2 min at 37°C in water bath. Thawed ovarium cortex strips were washed three times with a 37°C sterile L-15 medium for 5 min and stored in L-15 medium at 37°C until surgery was performed.

Ovarian Tissue Autotransplantation – Surgical Procedure

Frozen-thawed ovarian cortex strips were transplanted by laparoscopy or laparotomy. Firstly, two incisions were made on one of the ovaries, then a thawed ovarian fragment was carefully inserted under the cortex and was fixed by suture (Fig. 1). The procedure was repeated on the same ovary with another thawed cortex strip, then on the other ovary also with two thawed cortex strips.

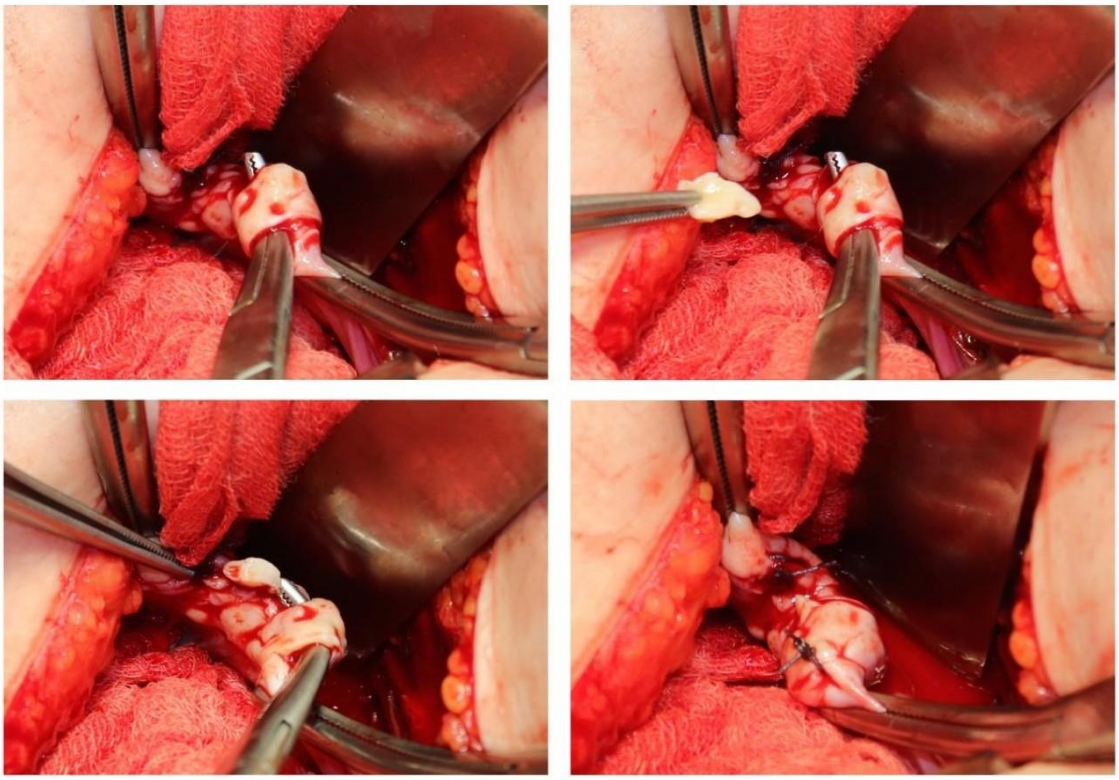

Figure 1: Steps of ovarian tissue autotransplantation: Preparation of ovarian cortex. Placing the implant taken from the freezing laboratory in subcortical pocket. Implant fixation. (images taken by Versys Clinics – Human Reproduction Institute)

RESULTS

Case 1

A 31-year-old nulliparous female, non-smoking, normal-weight patient has undergone routine cervical cancer screening which resulted in abnormal cytological findings (P5 HSIL). Following conization and fractional abrasion, histopathological examination of the sample confirmed the presence of cervical malignancy (histological diagnosis was carcinoma planocellulare). After the conization surgery, complete amenorrhea occurred (AMH at 5.9 ng/ml and FSH at 7.0 IU/l). The patient requested fertility preservation at our clinic and ovarian tissue cryopreservation was decided to be the best option for her. Surgery was performed after completing the informed consent and obtaining the permissions from the oncologists: the patient underwent laparoscopic removal of approx. 30% of ovaries on both sides, and a total of nine ultra-thin ovary cortex pieces were frozen by slow freezing and preserved in liquid nitrogen. An ovarian resection was sent for histopathologic analysis, which showed normal stroma, with no evidence of malignant involvement of the specimen. Subsequently, the patient received 50.4/1.8 Gy photon irradiation into the pelvic area, in parallel with the initiation of Cisplatin chemotherapy (40 mg/m2) 5 times, total daily dose of 70 mg, followed by 3x7 Gy HDR-AL boost. Two years after the therapy of the malignant disease, the control examinations were found to be negative. Three years after the cryopreservation, with MRI and PET-CT results indicating the remission of the malignancy, and oncologists reported no contraindication of tissue transplantation, two ovarian cortical strips were autotransplantated into each side of the remaining ovaries to alleviate menopausal complaints (AMH was <0.02 ng/ml and FSH was 47.9 IU/l before autotransplantation). Due to an excessive cervix scarring, the cervical canal was blocked, which we managed to drain from the direction of the uterus. After 10 days the inserted drain was removed. The patient was dismissed from the hospital after an uncomplicated postoperative period, and returned to her home in good general conditions. Follow-up is in progress.

Case 2

A 37-year-old nulliparous, non-smoking, normal-weight female patient presented in the hospital with hematochesia, where rectoscopy revealed an intraluminal bleeding mass. Past medical history was significant for Gilbert's disease only. Histopathology of the mass demonstrated invasive adenocarcinoma of the rectum with lymph node involvement. In parallel with the diagnosis, a benign liver tumor was identified incidentally, being most probably a focal nodular hyperplasia. The patient appeared in our clinic after the diagnosis and therapeutic plan. That time, her menstrual cycle was regular with an AMH level of 2.35 ng/ml and an FSH level of 8.1 IU/l. After consultation, ovarian tissue cryopreservation combined with controlled ovarian stimulation and oocyte freezing was decided as the best option for fertility preservation. Fertility preservation surgery was performed completing the informed consent and after that no contraindications from the oncologists were reported. The patient underwent laparoscopic removal of approx. 30% of ovaries on both sides, and a total of ten ultra-thin ovary cortex pieces were frozen by slow freezing and preserved in liquid nitrogen. A sample of the removed tissue was sent for histological analysis, which showed no evidence of malignancy affecting the ovaries. Subsequently, controlled ovarian stimulation was

performed whereby a mature egg was obtained and frozen. After fertility preservation, neoadjuvant radio-chemotherapy was applied, followed by laparoscopic Dixon operation and a stoma on the colon transversum, which was closed two months after surgery. Control PET-CT performed after the stoma closure was negative. The patient appeared at our clinic two years after the curative surgery for autotransplantation of the cryopreserved ovarian tissue. That time, the patient was amenorrhoeic with vasomotor symptoms, which status was confirmed by serum hormone measurements: AMH was <0.02 ng/ml and the FSH was 65.4 IU/l. After a repeated PET-CT, CT and colonoscopic control examinations – all showing negative result - as well as after obtaining oncological approval, two ovarian cortical pieces were autotransplanted into both remaining ovaries. During the operation, chromoperturbation was performed, showing a closed left Fallopian tube. After an uncomplicated postoperative period, the patient was admitted to her home in good general conditions.

Case 3

A 36-year-old nulliparous, non-smoking, normal-weight female patient was diagnosed with invasive ductal breast cancer five years ago. The patient has a past medical history of thoracic disc herniation, renal cyst and varicosity. Following diagnosis of the breast cancer, the tumor was surgically removed and adjuvant radio-chemotherapy was recommended. This was when the patient contacted our clinic, where, after signing the informed consent and after obtaining the oncological permissions, we performed a laparoscopic intervention, during which we have resected one-quarter of both ovaries for fertility preservation. A total of nine pieces of cortical ovarian tissue were frozen by slow freezing method. The procedure was followed by adjuvant radio-chemotherapy, after which the patient's menstrual periods became irregular, showing the signs of menopause. Follow-up CT, MRI and mammography were negative. Four years after the patient's therapy - when control PET-CT confirmed a tumor-free status - the patient was subjected to ovarian autotransplantation. The AMH value was 0.01 ng/ml and the FSH was 54.9 IU/l. During the operation we also performed chromoperturbation, which proved that the Fallopian tubes on both sides were closed. The patient was dismissed from our clinic after an uncomplicated postoperative period.

DISCUSSION

In the present paper we introduce the first three successful autotransplantation of frozen and cryopreserved ovarian tissue in Hungary. In all three cases, removal of ovarian tissue preceded the initiation of chemo- and radiotherapy and was stored for 2-4 years prior to autotransplantation. After complete recovery from the malignancy, all three patients showed the signs of premature ovarian insufficiency and menopausal symptoms.

According to international data, recurrence of ovarian function occurs within 3.5 to 6.5 months after autotransplantation, which is manifested by a decrease in FSH levels and an increase in estradiol levels. According to a study of 60 cases, ovarian function was restored in 93% of the patients following autotransplantation (11). Another study showed that the ovarian tissue was functional in 63% of patients within one year after transplantation (12). Pregnancy occurred in 29-33% of transplanted cases, about half of which spontaneously conceived, without the application of any assisted reproduction techniques. Thus, this method of fertility preservation is the only one that allows an eventual spontaneous conception. After transplantation, ovarian function is maintained usually for 5 to 10 years, depending on the age of the patient at the time of cryopreservation, on the gonadotoxic treatment and on the size of the tissue removed (13).

In the cases we have presented here, follow-up of patients are currently in progress. Hormone levels of the patients are continuously monitored after autotransplantation to determine whether tissue activity is restored. In all three patients, additional pieces of ovarian tissue are cryopreserved, allowing for re-transplantation once the currently transplanted tissue lost its function. Our cases suggest that freezing of ovarian tissue and subsequent autotransplantation may be a safe and quick option for fertility preservation.

The quality of life of patients recovered from a malignant disease is becoming increasingly important, with special emphasis on the question of fertility. Most recent ASCO recommendations underline the importance of proper orientation of patients with cancer. Patients should be informed of the potential gonadotoxic effects prior to the initiation of cancer therapy and of the available fertility preservation options. Interested patients should be referred to a reproduction specialist. Comprehensive information and the availability of fertility preservation options can reduce patient’s anxiety and improve quality of life (4).

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.V. was the principal investigator of the study and was involved in all aspects of patient care, surgical procedures and manuscript preparation. K.V. reviewed the literature and wrote the manuscript. L.L. was the oncologist specialist of the study. I.C. was involved in literature search and writing of the manuscript. Zs.B. was involved in surgical procedures. É.M. supervised the project, controlled the study protocol, indications, approvals, reviewed the literature and wrote the manuscript. All authors read and approved the final content of the manuscript.

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