Cell J
Cell Journal (Yakhteh)
Homepage: https://www.celljournal.org/
Case Report
Vol 25, No 11, November 2023, Pages: 809-812

Royan Institute First Attempts: Autotransplantation of Vitrified Human Ovarian Tissue in Cancer Patients

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Abstract

Today, timely diagnosis and therapeutic progress open a road of hope for survival in cancerous patients. Increased knowledge about the various cytotoxic treatment's impacts on ovarian function and fertility has resulted in a surge in the number of patients seeking to preserve their fertility before starting the anti-cancer treatment process. In this regard, embryo cryopreservation can be recommended for fertility preservation when the woman is married and has adequate time for ovarian stimulation. If patients are prepubertal girls or not married women, oocytes or ovarian tissue can be frozen instead to be used in the future. In this regard, the first attempts for ovarian tissue transplantations were conducted in 2016 and in 2019 for two cancerous patients whose ovarian tissue was cryopreserved in the Royan Human Ovarian Tissue Bank (Tehran, Iran). Unfortunately, the transplantations did not result in a live birth.

Keywords: Fertility Preservation, Human, Oncofertility, Ovarian Tissue, Transplantation

Citation: **Abtahi NS, Ebrahimi B, Ghaffari F, Fathi R, Valojerdi MR, Mehdizadehkashi A, Khodaverdi S, Yahyaei A, Faridi M. Royan institute first attempts: autotransplantation of vitrified human ovarian tissue in cancer patients. Cell J. 2023; 25(11): 809-812. doi: 10.22074/CELLJ.2023.2000360.1289**

Introduction

Because of timely diagnosis and new treatments, we encounter an increase in the survival rate for cancer. Increased knowledge about the various cytotoxic treatment impacts on the ovary has resulted in a surge in the number of patients seeking to preserve their fertility before starting treatments. If there is time for ovarian stimulation, embryo, and oocyte cryopreservation are standard techniques for fertility preservation. At present, ovarian cryopreservation is the only fertility preservation option that can be offered to women who have ovary stimulation limitations (such as inadequate time for stimulation or the impossibility of stimulating) and prepubertal girls. A transplantation of cryopreserved ovarian tissue after cancer treatment is a promising fertility restoration strategy that has already led to more than 200 live births worldwide (1-3).

After a decade of investigations, the Royan Human Ovarian Tissue Bank (OTB, ACECR, Tehran, Iran) was established in 2010 and started patient reception (4). In 2015, Royan Institute obtained international certificate, ISO 9001:2015, for this bank. From that time, consultations for approximately 1000 patients between 7- 47 years have been directed and ovarian tissues of more than 100 patients who had our criteria, have been cryopreserved.

In recent years, the Royan OTB has been requested for only three cases of ovarian tissue transplantation following cancer survival, that we will report two of them here. Before transplantation, a general consultation was conducted about the ovary transplantation and its outcomes in Iran and other countries. Consultation with an oncologist was conducted to declare the complete remission of the underlying disease and a consultation with the surgical team was carried out to manage possible adhesions during surgery for both patients. The informed consents were obtained from both patients (4).

Received: 19/April/2023, Revised: 04/July/2023, Accepted: 06/August/2023
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Case report

First case: She was a 22 years old girl with a history of colon cancer. She was affected by rectal bleeding when she was 17. The diagnosis of hyperplastic adenomatous polyps with focal high-grade dysplasia was established by colonoscopy biopsy. The pathological examination of the colon identified an invasive adenocarcinoma that its serosal surface and margin was tumor free, and also four reactive hyperplasia pericolic lymph nodes were detected. Her right ovary was resected and vitrified in 28 strips in the Royan OTB after one course of chemotherapy. Thereafter, she received five courses of chemotherapy and 40 sessions of radiotherapy. Hormone-replacement therapy (HRT), including 0.625 mg of conjugated estrogens (Aburaihan Pharma.Co., Tehran, Iran) plus 10 mg of medroxyprogesterone acetate (Aburaihan Pharma.Co., Tehran, Iran), was administered for the relief of menopausal symptoms monthly for 5 years. At the 5th year, she asked for the ovary transplantation. Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and Estradiol (E2) levels were measured before ovary transplantation (Table 1).

Second case: She was a 37-year old married woman who experienced a radical abdominal hysterectomy and bilateral salpingoophorectomy and pelvic lymph node dissection because of stage IA well-differentiated adenocarcinoma. She did not receive any chemotherapy or radiotherapy before ovariectomy. Both ovaries were resected, and 40 strips were vitrified and stored in the Royan OTB. Four years later, she asked for ovary transplantation. Serum concentrations of FSH, LH, and E_2 levels were measured before ovary transplantation (Table 2).

Ovarian tissue preservation procedure

In each case, after ovarian tissue resection, ovaries were quickly transferred to the Royan OTB (during approximately 1 hour) with a transfer medium at 4°C with ice packs. This medium consisted of Medium 199+Heppes (HTCM, Gibco, Paisley, UK) that was supplemented with 20% human serum albumin (HSA, Biotest, Germany). The transferred ovary was washed in the HTCM+20% HSA medium. The medullary part was removed and the thinnest cortical part was cut into 10×5×1 mm strips. Finally, the strips were vitrified in a two-step procedure as following:

First, each strip was transferred to an equilibration medium composed of HTCM, 7.5% ethylene glycol (EG, Sigma, St. Louis, MO, USA), 7.5% dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA), and 20% HSA for 15 minutes, and then the strips were washed in the vitrification medium [HTCM, 15% DMSO, 15% EG, 0.25 M sucrose, and 20% HSA] for 10 minutes. After the removal of the extra medium, the strips were directly transferred into liquid nitrogen. All steps were performed at 4°C (4).

Of note, one strip was randomly selected and fixed for histological and pathological evaluation via hematoxylin and eosin staining before cryopreservation (5).

Table 1: The hormonal profile of the first patient

Evaluation time according to transplantation day	FSH (mIU/mL)	LH (mIU/mL)	Estradiol (pg/mL)
Before transplantation	75.62	25.48	12
2 months after transplantation day	54	24.6	<5
4 months after transplantation day	64.8	27.8	37.2
5 months after transplantation day	57.3	30.9	52.3
7 months after transplantation day	92.6	32.7	13.7
9 months after transplantation day	159.6	71.7	<5

FSH; Follicle-stimulating hormone and LH; Luteinizing hormone.

Table 2: The hormonal profile of the second patient

Evaluation time according to transplantation day	FSH (mIU/mL)	LH (mIU/mL)	Estradiol (pg/mL)
Before transplantation	200	77	7
2 months after transplantation day	200	61	7
4 months after transplantation day	199	65	11
5 months after transplantation day	200	65	11
7 months after transplantation day	200	65	9.7
9 months after transplantation day	200	76	10

FSH; Follicle-stimulating hormone and LH; Luteinizing hormone.

Auto-transplantation

Both patients underwent the laparoscopic autologous orthotopic transplantation. The surgery and postoperative period were uneventful.

Before transplantation, the patient's ovarian cryopreserved strips were warmed and incubated at 37°C.

Warming procedure was performed in four steps in a descending sucrose concentration (1, 0.5, 0.25, and 0.125 M). The base medium was composed of HTCM and 20% HSA. To make sure tumor cells free, a histopathological examination of the storage tissue were performed before transplantation.

For the first case, 15 strips were warmed and prepared in two forms, including separate (7 strips) and ribbon-shaped (containing 8 strips). For preparing the ribbon-shaped, the strips were sutured under the surgical microscope by 8/0 "Coated VICRYL® (polyglactin 910) Suture - Ethicon" and a 1.5×2 cm ribbon was made. For transplantation, first, the pelvic area was evaluated well; no adhesion was observed, and the uterus was completely healthy and free from any pathology. Because the patient's right ovary had been removed and her left ovary was very small, a peritoneal pouch in the left broad ligament, under the fallopian tube was created. The ribbon-shaped strip was transplanted into the pouch, the medullary side facing the pelvic floor. Likewise, a peritoneal pouch was formed in the right broad ligament below the uterosacral ligament and the separate strips were transplanted inside it. Peritoneal pouch was sutured using a Vicryl suture. The patient was discharged 24 hours after surgery without any complications. The transplantation procedure for the first patient was conducted in the gynecology and obstetrics department of the Rasool_e_Akram University General Hospital, Tehran, Iran.

For the second patient, 17 strips were warmed. Nine separate strips were transplanted in the right ovarian fossa, and 8 separate strips in the left ovarian fossa. Under general anesthesia, her pelvic area was evaluated, and then an incision (1 cm) was made in the parietal peritoneum between the infundibular pelvic and uterosacral ligament on both sides. The sub-peritoneal pocket was bluntly dissected, and the ovarian strips were placed separately with their medullary side facing the pelvic floor. Finally, the peritoneal closure was performed with an interrupted suture (Monocryl suture 3-0, W3326, ETHICON surgical technology, USA). The transplantation procedure for the second patient was conducted in the gynecology and obstetrics department of the Royan Institute, Tehran, Iran.

Patients follow-up

Both patients' follow-up was carried out until 9 months after transplantation. Menstrual monitoring, ultrasonography, and measurement of the hormonal profile were conducted after the transplantation for the first patient. Her hormonal profile was reported in Table 1. Monthly sonography examinations revealed no follicular development and she didn't report menstruation during a year.

For the second patient, according to her hysterectomy and bilateral salpingoophorectomy, only hormonal profiles could be followed up which was reported in Table 2. Same as our first patient, no decrease in FSH levels and no increase in estradiol was observed, and as a result the transplanted ovarian strips are considered to be non-functional.

Discussion

If a transplantation is successful and its related transplanted ovarian tissue is functional, we must observe a decrease in the FSH level and an increase in the E2 level. Hormonal fluctuations are current after an ovarian tissue transplantation, in such a way that the FSH level will be decreased after 4-5 months of transplantation, and then will be returned to its premenopausal level (6). In the first patient, although 2-6 months after transplantation, the FSH hormone decreased to 50-60 mIU/mL, suddenly, an increasing trend was observed in the following months. Unfortunately, in the second patient, no decrease in the FSH level was observed after 9 months following transplantation.

As mentioned earlier, 200 live births have been reported worldwide but unfortunately we have not. It might be due to various reasons such as cryopreservation method, pre-cryopreservation ovarian tissue quality, follicle loss prior to the freezing process, size of the tissue, tissue revascularisation, and re-transplantation performance. Although, ovarian tissue integrity was well preserved by the vitrification method, only 2 live births have been reported after transplantation of vitrified tissue (7). Most live births were reported after a slow-freezing procedure (8-10). It seems that multiple transplantations, double or triple, may be successful in some patients (9). Although, we suggested a re-transplantation plan to both of our patients, they did not accept because of their personal desire. As there are no standard protocols for ovarian tissue cryopreservation and transplantation worldwide, differences in acquired results are predictable (11).

Conclusion

It is very encouraging that ovarian tissue cryopreservation and transplantation have resulted in a live birth. We acquired ovary cryopreservation knowledge in 2010 and tried transplantation 6 years later, but unfortunately did not result in a live birth. Continued research efforts are required to optimize our approach, and we hope to report successful transplantation soon.

Acknowledgments

We are thankful of financial support of the Royan Institute, Tehran, Iran. We are grateful of our participants and their kind collaboration. The authors declare that they have no conflict of interests.

Authors' Contributions

N.S.A., B.E., R.F.; Conducted ovarian tissue cryopreservation procedures. M.R.V.; Supervisor. F.G., A.M.K., S.K., M.F.; Autotransplantation surgery. F.G., N.S.A.; Patients follow up. A.Y.; Data collection. N.S.A., F.G., S.K.; Writing original draft of the manuscript. B.E.; Manuscript reviewer. All authors read and approved the final manuscript.

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