

Journal für

Reproduktionsmedizin und Endokrinologie

– Journal of Reproductive Medicine and Endocrinology –

Andrologie • Embryologie & Biologie • Endokrinologie • Ethik & Recht • Genetik
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Transplantation of Frozen Thawed Ovarian Tissue – State of the Art

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J. Reproduktionsmed. Endokrinol 2013; 10 (Sonderheft
1), 55-58

www.kup.at/repromedizin

Online-Datenbank mit Autoren- und Stichwortsuche

Offizielles Organ: AGRBM, BRZ, DVR, DGA, DGGEF, DGRM, D-I-R, EFA, OEGRM, SRBM/DGE

Indexed in EMBASE/Excerpta Medica/Scopus

Krause & Pachernegg GmbH, Verlag für Medizin und Wirtschaft, A-3003 Gablitz



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Transplantation of Frozen Thawed Ovarian Tissue – State of the Art

K. T. Schmidt¹, E. Ernst², T. Greve³, C. Y. Andersen³

Worldwide, an increasing number of cancer patients have some of their ovarian tissue cryopreserved for fertility preservation purposes prior to treatment of a malignant disease. The purpose of this review is to summarize the results from ovarian tissue transplantation and the different techniques that can be applied when autotransplanting the tissue. To date, a total of 18 babies have been born as a result of cryopreserved/thawed autotransplanted ovarian cortical tissue and an even larger number of premenopausal women have regained their ovarian function and menstrual cyclicity as a result of autotransplantation. Orthotopic or heterotopic sites can be chosen for the cortical grafts, but so far all babies born have been from orthotopic graft sites. Follow-up studies after transplantation have shown encouraging results regarding the longevity of the grafts with up to 8 years of graft viability. Reassuringly, no cases of introduction of the original disease have so far been reported in cancer survivors grafted with frozen/thawed ovarian tissue. **J Reproduktionsmed Endokrinol 2013; 10 (Special Issue 1): 55–8.**

Key words: cancer, cortex, cryopreservation, fertility preservation, ovary

■ Introduction

The 5-year survival rate after many cancers has improved significantly over the past decades, especially regarding childhood cancers and cancers in the young adults [1]. As a consequence, one in 593 adults will be a survivor of a childhood cancer [2] and this can be attributed to the more aggressive chemotherapy regimens and the targeted radiotherapy treatment that are used today. A paradox exists, however, between the wanted destruction of the malignant cells and the unwanted side effects that may arise due to destruction of benign cells. For younger women in their fertile years a most unwanted and serious side effect to cancer treatment is the loss of ovarian function that occurs if all the ovarian follicles are destroyed. This is a known side effect to both chemotherapy, especially if the protocol includes an alkylating agent, and to abdominal radiation therapy [3, 4]. For many young women who have not yet attempted to conceive before they are diagnosed with cancer, this risk of infertility is very serious and alarming and for this reason, several options of fertility preservation have been developed and are offered to more and more young women before the initiation of their cancer treatment. Cryopreservation of ovarian tissue is one method of fertility preservation, which has been developed and refined primarily over the last decade [5]. An entire ovary, a semi-ovary or ovarian cortical biopsies are

collected and cryopreserved for later use. If the woman as a consequence of her treatment experiences premature ovarian insufficiency (POI) she can request the frozen ovarian tissue thawed and transplanted. This has been performed on many cancer survivors worldwide and the majority of these have regained their ovarian function after transplantation [6, 7] and so far a total of 18 children have been born as a result from transplantation [8–20].

■ Effect of Chemo- and Radiation Therapy on the Ovary

Different chemotherapeutic agents exert different mechanisms of action on the cells and on the follicles in the ovary. The alkylating agents act on both resting and dividing cells and the quiescent, meiotically inactive oocytes in the primordial follicles are more susceptible to the damaging effects of alkylating agents than other chemotherapeutic agents. This has been found in a study by Meirow, who compared the risk of POI in young cancer patients according to which drugs they received. Administration of alkylating agents had an OR of 4.0 for POI, which was a significantly higher risk than when platinum agents (OR = 1.8), plant alkaloids (OR = 1.2), or antimetabolites (OR < 1) were used [21]. Oocytes are very susceptible to the damage caused by radiotherapy [4] and the majority of patients receiving a dose

> 20 Gy will become sterile as a consequence [22]. Treatment with bone marrow transplantation (BMT), which is often used in leukaemia patients or patients with non-malignant haematological conditions such as Thallasaemia or aplastic anemia, causes loss of ovarian function in most patients [23] as a consequence of the pre-conditioning protocols consisting of high-dose chemotherapy and total body irradiation.

■ Cryopreservation of Ovarian Tissue

By laparoscopy, ovarian tissue can be excised on a short notice, usually without any significant delay of a potentially gonadotoxic treatment. Cryopreservation of ovarian tissue involves removal of an entire ovary or parts of an ovary prior to treatment. The ovarian cortex, which harbours the primordial follicles, is isolated in a thickness of approximately 1 mm. After appropriate equilibration in a cryoprotectant medium the tissue is frozen and stored in liquid nitrogen and can potentially be kept frozen for many years [24]. When the woman has been cured she can have some of the pieces of tissue thawed and transplanted, if she has become menopausal as a consequence of her treatment. Some of the primordial follicles within the pieces of cortical tissue will survive the freezing and transplantation procedure and have the capacity to be reactivated and start to grow and thus re-establish a cyclic endo-

Received: June 6, 2012; accepted: September 11, 2012

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Table 1. 18 Live Births after Transplantation of Frozen-Thawed Ovarian Tissue

Age at Cryo-preservation (Years)	Disease	Gestation (Weeks)	Sex	Weight (kg)	Reference
25	Hodgkin's Lymphoma	39	F	3.720	Donnez, 2004 [8]
17	Neuroectodermic Tumor	38	M	2.830	Donnez, 2011 [17]
28	Non Hodgkin's Lymphoma	38	F	3.000	Meirow, 2005 [9]
24	Hodgkin's Lymphoma	41	F	3.130	Demeestere, 2007 [10]
		39	F	2.870	Demeestere, 2010 [16]
27	Ewing Sarcoma	39	F	3.204	Andersen, 2008 [11]
		39	F	3.828	Ernst, 2010 [15]
		41	M	4.015	Andersen, 2012 [37]
25	Hodgkin's Lymphoma	37	M	2.600	Andersen, 2008 [11]
20	Hodgkin's Lymphoma	38	M	3.089	Silber, 2008 [12]
27	Microscopic polyangiitis	37	F	2.030	Donnez, 2011 [17]
36	Breast Cancer	33	M	1.650	Sánchez-Serrano, 2010 [14]
		33	M	1.830	
20	Sickle Cell Anemia	38	F	3.700	Roux, 2010 [13]
19	Thalassemia	39	M	3.026	Revel, 2011 [18]
n. s.	Hodgkin's Lymphoma	n. s.	n. s.	n. s.	Silber, 2012 [20]
n. s.	Premature Ovarian Failure	n. s.	n. s.	n. s.	Silber, 2012 [20]
25	Hodgkin's Lymphoma	38	M	3.360	Dittrich, 2012 [19]

n. s.: not stated, M: male, F: female

crine milieu needed for a normal menstrual cycle and conception.

■ Orthotopic Transplantation

Orthotopic transplantation means transplanting tissue into its normal place in the body, which, in the case of ovarian tissue, means grafting it into the remaining ovary or at the site of the removed ovary. So far, all the children that have been born as a result of ovarian tissue transplantation originate from orthotopically grafted tissue. Table 1 gives an overview of the 18 children that have so far been born following autotransplantation of cryopreserved/thawed ovarian tissue. The first report came from Donnez' group in Belgium in 2004 [8]. They transplanted strips and small cubes of cryopreserved/thawed ovarian cortical tissue to a small peritoneal window, which was created by laparoscopy 7 days earlier, beneath the right ovarian hilus. This was repeated after 4 months. After 10 months the patient conceived spontaneously and later gave birth to a healthy baby girl. Later, other groups followed reporting successful pregnancies in cancer patients after orthotopically transplanted tissue into the remaining ovaries as either cortical strips or fragments [9–12, 14–16] or tiny ovarian fragments im-

mersed in oocyte wash buffer [9], although in the latter method ovarian function never resumed. Other orthotopic grafts sites have been introduced such as the broad ligament or a peritoneal pocket close to the broad ligament [18, 19] or a peritoneal pocket between the iliac vessels [13]. In theory, when grafting to an orthotopic site, the patient should be able to conceive naturally and so far, the majority of the babies born following this procedure have resulted from spontaneous conceptions. But some women will need in vitro fertilization, IVF, in order to become pregnant, either because they already had a history of subfertility before their cancer diagnosis or for other reasons such as a partner with a low sperm count.

■ Heterotopic Transplantation

So far, no children have been born following transplantation to a heterotopic transplantation site. In our group, we have had 2 biochemical pregnancies arising from oocytes aspirated from a graft site in the anterior abdominal wall, but unfortunately these pregnancies never developed further [7]. But the fact that IVF led to the aspiration of mature metaphase II oocytes that were fertilized

and able to implant, although only briefly, means that oocytes deriving from cryopreserved/thawed ovarian tissue are able to undergo a normal maturation process and there is no reason to believe that the pregnancies reported after transplantation have not arisen from the grafted tissue. Oktay and co-workers used a heterotopic graft site for their first autotransplantation. Two women with cervical cancer and recurrent benign ovarian cysts, respectively, had some of their ovarian tissue transplanted subcutaneously to the forearm [25]. After 10 weeks and 6 months, respectively, a follicle appeared at the graft site and levels of FSH normalized. Percutaneous oocyte aspirations yielded a mature oocyte. The same author later transplanted cryopreserved/thawed ovarian tissue below the skin of the abdomen but several IVF attempts only yielded three metaphase II oocytes from a total of 20 follicles [26]. Kim transplanted ovarian tissue into the space between the rectus sheath and the rectus muscle in five cancer survivors, who all regained their ovarian function between 12–20 weeks after transplantation [27]. We have transplanted fragments of cortical tissue into a peritoneal pocket corresponding to the abdominal wall between the umbilicus and the pubic bone. After IVF this graft

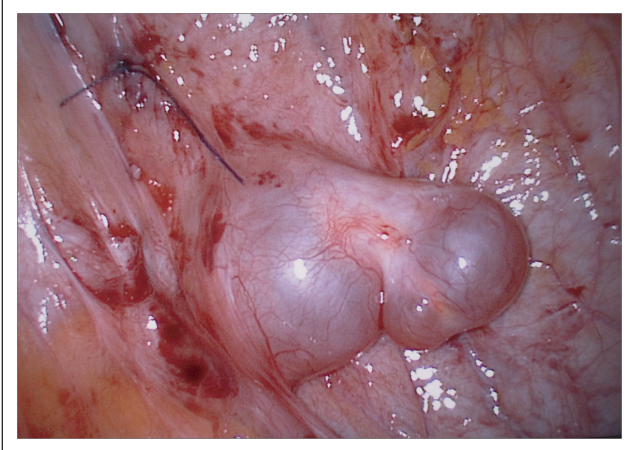


Figure 1. Spontaneous follicular development from the first graft site in a peritoneal pocket at the pelvic wall above the internal iliac artery.

site has yielded mature oocytes and in spontaneous cycles the site has revealed pre-ovulatory follicles as well [7].

■ Transport of Ovarian Tissue Prior to Cryopreservation

There is no doubt that cryopreserving ovarian tissue is a specialist task that should ideally be centralised to only a few centres with a sufficient number of cases to maintain skills and a high expertise. This facilitates quality control, proper equipment and personnel that maintain a proper expertise and can fulfil all the clinical, legal and scientific standards that are required for appropriate conduction of the procedure. However, this still allows other centres to offer the initial counselling and the collection of the tissue. We have previously shown that ovarian tissue remains viable after transport of up to five hours cooled on ice prior to freezing [28] and shown in another case that the tissue remained viable after a 20 hour period on ice prior to freezing [24]. In fact, all of the 4 children so far born in Denmark originate from tissue that has been transported for up to 5 hours before cryopreservation. Recently, Dittrich and co-workers reported of a live birth after ovarian tissue autotransplantation, where the tissue had originally been transported overnight and had been kept cool for more than 20 hours before it was cryopreserved [19]. This should encourage centres that do not offer ovarian tissue cryopreservation to enter collaboration with a centre with the required expertise and still offer this procedure to their patients, as the tissue does not need to be cryopreserved as soon as it has been collected.

■ Risk of Reintroduction of the Original Disease after Transplantation

In cancer patients it cannot be excluded that the tissue harvested before cancer treatment may harbour malignant cells. If the ovarian tissue is infiltrated with malignant cells there may be a risk of introducing a sufficient number of malignant cells to cause a relapse. For certain cancer types this risk is higher than for others. Ovarian tissue from leukaemia patients, for instance, has a high risk of harbouring leukaemic cells [29, 30] and so far no patients cured of leukaemia have been offered ovarian tissue transplantation due to the risk of relapse. Other cancers have a much lower risk of malignant cell contamination in the ovaries, especially if the ovary is cryopreserved in patients with local disease only. It has been estimated that worldwide approximately 45 patients with a previous cancer diagnosis have had ovarian tissue transplantation and so far there have been no reports of relapse due to the grafted tissue. This is reassuring, but further studies on the safety are warranted. So far studies on breast cancer patients and lymphoma patients have shown a very low risk of malignant cell infiltration in the cortical tissue from these patients [31–34].

■ Our Experience

In Denmark, a total of 22 patients have so far received transplantation of frozen/thawed tissue a total of 31 times. Thus, 9 patients have received one additional transplant, either to augment the follicle pool and thus their chances of becoming pregnant or because the function of the

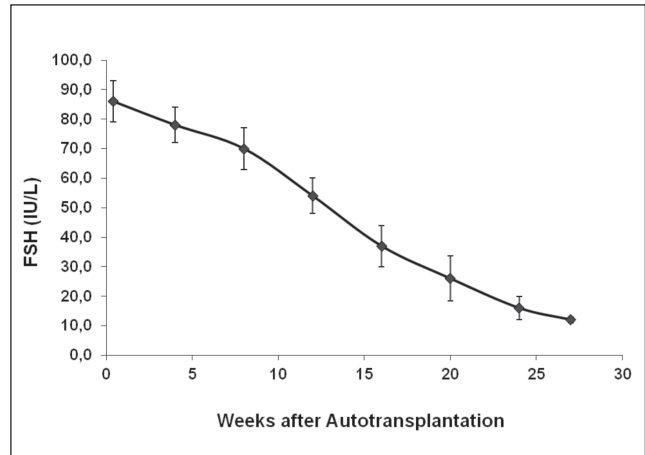


Figure 2. Mean concentration of FSH (IU/L) ± SEM following transplantation of frozen/thawed ovarian tissue to 15 Danish women.

first grafted tissue was exhausted and the patient had become menopausal again. Figure 1 shows spontaneous follicular development corresponding to the first graft site during a second autotransplantation just above the first graft in a peritoneal pocket above the internal iliac artery. All 22 patients resumed endogenous hormone production and follicular development after transplantation. Levels of follicle stimulating hormone (FSH) that were high before transplantation decreased gradually and after approximately 20 to 25 weeks a preovulatory follicle appeared on ultrasonography and the patient resumed menses. Figure 2 shows the mean concentration of FSH (IU/L) ± SEM following transplantation. Apart from a 13-year-old girl, who had her tissue transplanted in order to induce a natural puberty and three other adult cancer survivors who wanted their tissue transplanted to alleviate menopausal symptoms, all of our patients initially requested transplantation because of a pregnancy wish. So far, 6 women have experienced a total of 9 pregnancies after transplantation leading to 2 biochemical pregnancies, 2 first trimester spontaneous abortions, one induced abortion [35] and the delivery of 4 healthy babies. One patient, who was diagnosed with Ewing's sarcoma in 2004, has had 3 babies after transplantation of some of her cryopreserved/thawed tissue in 2005. Her first pregnancy was the result of IVF but the other 2 children were spontaneously conceived [11, 36]. The other patient to deliver a child had been treated with BMT for Hodgkin's disease and conceived after several attempts of IVF and a second transplantation and delivered a healthy boy [11].

The duration of the transplants in our series has varied. In general, the age of the patient at the time of cryopreservation is a determining factor on the longevity of the graft; i.e. the younger the patient at the time of cryopreservation the longer the graft will last. Other determining factors are the amount of tissue that is transplanted and whether or not the patient has received any chemotherapy prior to collection of the tissue. Most patients in our series have experienced at least 2–4 years of activity with the tissue still functional. One patient had her first transplant in 2004 and a second transplant in 2008 while the first graft was still functioning and still has functional tissue 8 years after the first transplant. Additionally, most of the patients in our series still have tissue in the freezer for 1 or 2 more transplants after their first grafts stop functioning.

■ Fertility After Cryopreservation of an Ovary and Cancer Treatment

We have recently conducted a questionnaire study looking at the ovarian function and fertility after treatment of a malignant disease in women with one ovary due to cryopreservation of the other. Fortunately, not all women experienced POI after their potentially gonadotoxic treatment. In fact, we found that only 21% of 143 responders stated that they had become menopausal after treatment. In the remaining women with an intact ovarian function those with a pregnancy wish succeeded in becoming pregnant and giving birth to healthy children in the majority of the cases [37].

■ Conclusion

Although still considered experimental, cryopreservation of ovarian tissue has proved to be a viable way of restoring ovarian function after cancer treatment and with 20 babies born worldwide, the results are promising. For many women facing a potential gonadotoxic treatment and a risk of POI, cryopreservation of ovarian tissue offers a hope of keeping her fertility in the future and her own endogenous hormone production and for many cancer patients this is a relief during an otherwise difficult period of their lives. It is very likely that in the future, fertility preservation will be an integrated part of the cancer treatment ad-

ministered to young cancer patients thus improving their quality of life and reducing one of the potential side effects of cancer treatment: infertility.

■ Conflict of Interest

No potential conflict of interest to this article was reported.

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