

Cryopreservation and Autotransplantation of Ovarian Tissue in Cancer Patients: Is It Safe?

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ONCOLOGICAL THERAPY may severely compromise the future fertility of girls and young women with cancer and thereby limit their quality of life. Various strategies to preserve fertility before the start of gonadotoxic treatment have been proposed, such as *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), vitrification of oocytes, and cryopreservation of ovarian tissue.¹ Unfortunately, not all of these techniques are suitable for pre-pubertal girls and adolescents. Not only do IVF and ICSI require a male partner or donor sperm for fertilization, procedures using gonadotrophin administration and oocyte retrieval are considered inappropriate for sexually immature patients.²

In case of ovarian tissue cryopreservation, neither hormonal stimulation nor a stable relationship is necessary. These characteristics make ovarian tissue cryopreservation especially suitable for adolescents and the only option available for fertility preservation in pre-pubertal girls.^{1,2} Furthermore, as ovarian tissue can be obtained directly after diagnosis, there is only minimal interference with cancer treatment.¹

The main aim of cryopreservation of ovarian tissue is to restore reproductive potential by retransplanting the tissue. This can be performed once the patient has overcome her disease and wishes to conceive but experiences premature ovarian insufficiency due to the cancer treatment.^{1,3} Although the technique of ovarian tissue autotransplantation and subsequent autografting is still considered experimental, 16 live births have already been reported; 12 of these children were born to 9 women who survived cancer.^{3,4}

As no central registration for ovarian tissue cryopreservation or autotransplantation exists, the total number of procedures carried out worldwide is unknown.⁵ Nevertheless, a considerable number of cryopreservation and autotransplantation procedures have been reported,^{6–14} indicating that cryopreservation of ovarian tissue have been performed on a large scale in the past decade. Therefore, the number of cancer survivors requesting autotransplantation of their

ovarian tissue is anticipated to increase considerably in the near future.⁵

Although autotransplantation procedures are already being performed in oncological patients worldwide, the risks of recurrent malignancy due to transmission of cancer cells via the ovarian graft still remains largely unknown. This information is critical for proper counseling and clinical decision making for cancer survivors considering autotransplantation, as well as for newly diagnosed oncological patients.^{1,5} Here, we discuss ovarian tissue autotransplantation-related safety issues and make recommendations for future research and patient counseling.

Why Is There Concern About the Safety of Ovarian Tissue Autotransplantation?

The alarm was raised on the safety of ovarian tissue autotransplantation in cancer survivors for the first time in 1996. At that time, healthy mice transplanted with fresh or cryopreserved ovarian tissue from mice with lymphoma developed the disease and died, though one mouse that received cryopreserved tissue remained healthy.¹⁵ A later xenotransplantation study also showed that acute lymphoblastic leukemia was transmitted to recipient animals via human ovarian grafts.¹⁶

By using histology or polymerase chain reaction (PCR), cancer cells have been detected in the ovaries from patients with leukemia and Ewing sarcoma.^{16–18} Apart from these findings, it is known from clinical experience that different types of oncological diseases have the potential to metastasize to the ovaries. For example, breast cancer, a common indication for ovarian tissue cryopreservation,^{9,11–13} has been repeatedly demonstrated to metastasize to the ovaries.^{19,20}

In contrast to these alarming results, other studies report more reassuring findings when it comes to the safety of ovarian tissue autotransplantation. Several studies failed to show the presence of malignant cells in ovarian cortical tissue

from patients with breast cancer,^{21,22} lymphoma,^{23,24} and various other oncological diseases.²⁵

Several authors have tried to provide guidance for clinical decision making and counseling by classifying different oncological diseases as having a low, intermediate, or high risk of ovarian involvement based on the literature available.^{26–28} However, the absolute magnitude of the risk of ovarian metastasis remains unclear, with some diseases being classified in different risk categories in various publications.

How to Assess the Risk of Oncological Relapse due to the Reintroduction of Tumor Cells via Transplantation

One of the pivotal issues when assessing this risk of relapse is the chance that malignant cells derived from a certain tumor type and stage are present in the ovaries at the time ovarian tissue is cryopreserved. Therefore, an overview of epidemiological data on ovarian metastasis in different primary tumor types, as well as the use of valid diagnostic tools with which the involvement of the ovaries can be assessed in each individual cancer patient, would be useful.

Histology and polymerase chain reaction

Minimal residual disease in ovarian tissue from cancer patients can be detected by using histology and/or PCR.^{16–18,22–25} During histological examination, the presence of cancer cells may go unnoticed.¹⁸ PCR is much more sensitive than histology, but tumor-specific PCRs are available for only a limited number of oncological diseases. In addition, a positive PCR signal does not provide information about the viability of tumor cells present in the positive tissue, nor about their ability to cause relapse after transplantation.

Cortical strips from the same ovary analyzed by PCR may give different results with respect to the detection of cancer cells, indicative of sampling bias.¹⁸ As the ovarian strips that are analyzed by histology or PCR are no longer available for transplantation purposes, it remains uncertain whether the strips that are actually used for transplantation purposes are indeed devoid of cancer cells.

In conclusion, histology and PCR may provide information about the incidence of minimal residual oncological disease in the ovarian tissue, but do not guarantee that transplantation is safe.

Xenotransplantation

Prior to performing an autotransplantation of ovarian tissue to the human recipient, one or a few cortical fragments may be xenografted to a suitable immunodeficient host animal.^{15,16,23} Should the recipient animal develop malignant disease, the tissue obviously contains tumor cells, as with the mice that developed intraperitoneal leukemic masses after being grafted with frozen-thawed ovarian tissue from patients with leukemia.¹⁶ However, if the animals remain healthy, this still offers no guarantee for safe autotransplantation, as there is again inevitable sample bias.²⁹ In addition, growth of the cancer cells might be different in the humans and immunocompromised animals.

Follow-up after autotransplantation of ovarian tissue

The duration of follow-up after autotransplantation of ovarian tissue is still relatively short, and transplantations

have not yet been performed on a large scale.^{3,5,8} Therefore, the safety of autotransplantation cannot be ensured by the follow-up data currently available. Finally, one should consider that the available follow-up data might be biased by selective reporting, as no central registration exists.⁵

Epidemiological data on the incidence of ovarian metastases

Follow-up of patients with a certain type of tumor or reports from prophylactic oophorectomy during tumor resection may provide information about the incidence of ovarian involvement. However, cancer patient follow-up studies investigating the incidence of ovarian involvement do not always clearly describe how they assessed the presence of ovarian involvement; tumor stage, prior treatment, and other important prognostic variables may also not be described. With such a large number of confounding factors left unspecified, it is quite difficult to extract useful information from this type of report. Therefore, studies should provide clear information about tumor types, stages, patient characteristics, and other relevant prognostic factors, as well as details of the research methods used. Studies meeting these strict criteria may be scarce, and a systematic literature search for these reports is strongly advisable.

One of the difficulties in interpreting epidemiological data is that these reports include patients who did not have their ovarian tissue cryopreserved. This means that for a large number of cases, their ovaries have been exposed to radio- or chemotherapy that may have eliminated minimal residual disease in this organ. This is generally not the case in patients who did opt for ovarian tissue cryopreservation. In addition, although clinical studies may provide information about the incidence of clinically relevant ovarian metastases during the follow-up period, they do not inform about the percentage of patients who have minimal ovarian involvement at the time of cancer diagnosis. As a result, it is difficult to estimate the significance of a single tumor cell in cryopreserved ovarian tissue, as its capacity to develop into a metastatic lesion after autotransplantation is unknown.

Conclusion and Recommendations

In conclusion, the available information for counseling cancer survivors with regard to their risk of oncological relapse after ovarian tissue autotransplantation is insufficient. In addition, data from different types of studies cannot be interpreted unambiguously.

It is clear that data on the risk of reintroducing tumor cells with ovarian transplant are necessary, as a growing number of patients are expected to request cryopreservation and autotransplantation in the near future. We would therefore like to make the following recommendations.

First, we propose the creation of a database in which all cancer-related cryopreservation and autotransplantation cases are registered, including follow-up data. Registration should preferably take place on an international level. The European Society of Human Reproduction and Embryology (ESHRE; Task Force Fertility Preservation), the International Society for Fertility Preservation (ISFP), or the American Society for Reproductive Medicine (ASRM) may provide the necessary infrastructure for such a registry, and would be logical candidates for setting up this initiative. The

anonymous data from the resulting database should be available to all clinicians who counsel cancer patients on fertility preservation, ensuring that unbiased follow-up data will be available as soon as possible.

Next, we suggest performing additional research to improve knowledge and introduce new options into clinical practice. Studies focusing on the diagnosis and clinical significance of minimal residual disease in the ovary will be pivotal. In addition, further research is needed on techniques that circumvent the risk of reintroducing cancer cells via autotransplantation, such as xenotransplantation of ovarian tissue followed by IVF, *in vitro* maturation (IVM),^{1,30} and oocyte formation from stem cells.³¹ To date, autotransplantation of ovarian tissue is the only clinically available option to restore fertility after ovarian tissue cryopreservation.¹ For current patient counseling, a systematic review of the available data on ovarian involvement in neoplastic disease is therefore urgently needed. Together with the follow-up data from autotransplantations, this review will facilitate counseling for today's patients who are confronted with fertility preservation choices.

These recommendations should lead to improvements in our ability to select the most suitable fertility preservation option for each individual patient. Until then, we strongly feel that both clinicians and patients should be aware of the uncertainties regarding autotransplantation safety.

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