Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue

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Ovarian tissue cryopreservation and transplantation is a real option to preserve and restore fertility in young cancer patients. However, there is a concern regarding the possible presence of malignant cells in the ovarian tissue, which could lead to recurrence of the primary disease after reimplantation. A review of the existing literature was done to evaluate the risk of transplanting malignant cells in case of the main malignant indications for ovarian tissue cryopreservation. For ovarian tissue from patients with hematologic malignancies, it is of paramount importance to identify minimal residual disease before ovarian tissue transplantation. Indeed, these pathologies, reviewed here in detail, are considered to be most at risk of ovarian metastasis. (Fertil Steril® 2013;99:1514–22. ©2013 by American Society for Reproductive Medicine.)

Key Words: Ovarian tissue cryopreservation, ovarian tissue transplantation, cancer, risk, malignant cells

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Cryopreservation of ovarian tissue is the main option available to preserve fertility in women who require cancer treatment but cannot delay the start of chemotherapy and in prepubertal patients.

In our department, hematologic malignancies are the most common indication for ovarian tissue cryopreservation, representing 37.5% of all indications (1). In this category, Hodgkin lymphoma (HL) is the most frequent, followed by leukemia and then non-Hodgkin lymphoma (NHL). According to the current SEER data (NCI, USA, http://seer.cancer.gov; Surveillance, Epidemiology, and End Results), the most common hematologic cancer in females under the age of 20 years is leukemia, followed by HL and then NHL.

Reversing treatment-related premature ovarian failure with autotransplantation of frozen-thawed ovarian tissue is now becoming a reality, with 24 live births reported so far using this technique (2).

It is expected that in the near future, more and more cancer patients cured of their disease will request reimplantation of their cryopreserved ovarian tissue. However, there is concern regarding the possible presence of malignant cells in the ovarian tissue, which could lead to recurrence of the primary disease after reimplantation (3).

To increase the safety of ovarian tissue transplantation, identification of tumor involvement in the ovaries and detection of the presence of cancer cells in ovarian tissue is of paramount importance (3).

The aim of this review is to examine all available evidence of the risk of transplanting malignant cells with frozen-thawed ovarian tissue from cancer patients. The risks are evaluated for each of the main indications for ovarian tissue cryopreservation.

LEUKEMIA

In leukemia, malignant cells may be present in the bloodstream, and therefore also in the ovaries, so there may be a risk of transferring diseased cells with the ovarian tissue. A study by

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Jahnukainen et al. (5) showed that transplantation of testicular cells from leukemic donor rats transmits acute leukemia to healthy recipients. For ovarian tissue from hematologic cancer patients, it is of great importance to identify minimal residual disease (MRD) before ovarian tissue transplantation (3, 6).

Meirow et al. (3) were the first to detect chronic myeloid leukemia (CML) cells in frozen-thawed ovarian tissue from a patient with the disease by real-time quantitative polymerase chain reaction (RT-qPCR), which proved to be positive for BCR-ABL transcripts, and therefore excluded transplantation of the stored ovarian tissue. A recent retrospective analysis of 5,571 autopsy findings of female patients under the age of 40 years in Japan found, by histology, leukemic involvement of the ovaries in 8.4% of leukemia patients (7). Very little has been published on the incidence of ovarian metastasis in CML. In case of acute lymphoblastic leukemia (ALL), ovarian metastases have been found in up to 30% of patients at autopsy, but are rarely detected clinically (8).

In recent studies, molecular biology was used to evaluate the presence of leukemic cells in cryopreserved ovarian tissue from patients with CML, acute myeloid leukemia (AML), and ALL, leukemia types that are the most frequent indications for cryopreservation of ovarian tissue (1, 9–12).

Ovarian Tissue Histology

In the above-mentioned studies, histology and immunohistochemistry failed to detect malignant cells in fresh or frozen ovarian tissue from any leukemia patients. Indeed, samples were analyzed by experienced pathologists, and no malignant cells were found in ovarian tissue of patients with any form of the disease (ALL, CML, or AML). Moreover, ovarian follicles were present in all the samples. Nevertheless, we should stress that since these publications, malignant cells have been detected by histology/immunohistochemistry in three cases of ALL (1).

Polymerase Chain Reaction Analysis

Determination of BCR-ABL fusion gene transcripts. The Philadelphia chromosome is pathognomonic for CML. It is the result of reciprocal translocation t(9;22), which brings the ABL gene from chromosome 9 together with the BCR gene from chromosome 22, creating a fusion gene known as BCR-ABL. Tumor-specific breakpoint cluster region/proto-oncogene tyrosine protein kinase ABL1 (BCR–ABL1) transcripts can be detected with use of RT-qPCR (3, 13). Molecular detection of leukemic cells in ovarian tissue can always be carried out for CML, because the presence of the BCR-ABL fusion gene is characteristic of the disease.

Other specific translocations [e.g., t(1;19)] can be present in ALL disease. In MRD studies, PCR analysis at the time of diagnosis was performed on blood and bone marrow, and the same markers were used on frozen-thawed ovarian tissue.

Detection of clonal immunoglobulin and T-cell receptor gene recombinations. MRD in case of ALL is evaluated by RT-qPCR analysis of leukemia-specific junctional regions of rearranged immunoglobulin (Ig) genes and T-cell receptor (TCR) genes, which can be considered as DNA fingerprints of leukemic cells (14). For ALL, genetic markers are not always detected. Recurrent chromosome translocations are reported in 38% of ALL patients (15, 16). If no chromosomal abnormalities are evidenced in blood or bone marrow of ALL patients, specific Ig gene rearrangements can often be tested, even if the sensitivity of these tests is lower. Nevertheless, some ALL cases do not display any markers at all, and so far there are no available molecular methods to evaluate the risk of contamination by malignant cells.

PCR products obtained from Ig and TCR gene rearrangements were thus analyzed by gene scanning, as described by Van Dongen et al. (17).

The present review reports both the Belgian and Danish experience in the field of ovarian tissue reimplantation in leukemia patients.

THE BELGIAN EXPERIENCE (DOLMANS ET AL., BLOOD 2010) (9)

Is there a risk of malignant cell contamination when ovarian tissue is retrieved from patients with leukemia in the active phase?

CML and ALL

In vitro study. Among CML patients (n = 6), two were found to be positive for the BCR-ABL leukemic marker in their ovarian tissue (patients 1 and 3; Table 1). All CML patients received hydroxycarbamide with or without imatinib before ovarian tissue cryopreservation. Cryopreservation was carried out as bone marrow transplantation (BMT) was scheduled in these patients. Amplification curve results for the BCR-ABL fusion gene showed a positive signal in the bone marrow of diseased patients at diagnosis and also in their frozen-thawed ovarian tissue (Table 1).

Among ALL patients (n = 12), two were excluded from PCR analysis because no molecular markers were available. Of the remaining ten, seven showed positive molecular markers in their cryopreserved ovarian tissue. One was positive for the BCR-ABL fusion gene, the second for t(1;19)(q23;p23.3), and the other five for Ig and/or TCRγ rearrangement genes (Table 1).

Among the seven patients who were positive for ALL markers in their ovarian tissue, four had not received any chemotherapy before ovarian tissue cryopreservation (patients 8, 11, 13, and 14) and three had already undergone one regimen of chemotherapy (patients 7, 10, and 18).

In conclusion, using disease-specific PCR techniques, the authors found contamination of ovarian tissue in 33% and 70% of CML and ALL patients, respectively (9).

In case of CML with indications for BMT, hematologists and oncologists have time to send their patients to the gynecologist for fertility preservation options such as ovarian tissue cryopreservation. In Dolmans et al.'s series (9), all CML patients underwent ovarian tissue cryopreservation before sterilizing chemotherapy, but after receiving hydroxycarbamide with or without imatinib. Nevertheless, we should stress that even after a minimum of 6 months of this treatment, PCR results showed that ovarian tissue was still positive (patients 1 and 3).