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FERTILITY PRESERVATION

The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature

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Abstract
Background Transplantation of frozen/thawed ovarian tissue from patients with a malignant condition is associated with a risk of re-introduction of the disease as the tissue usually is removed before anti-cancer therapy and may thus contain malignant cells. We review studies investigating the presence of malignant cells in cryopreserved ovarian tissue from patients with malignant disease and based on the strength of the evidence, recommendations for transplantations are proposed.
Materials and methods A systematic review of the literature. All peer reviewed studies evaluating the presence of malignant cells in cryopreserved human ovarian tissue were included. Data were searched in Pubmed and Embase with no language restrictions.
Results The majority of the reviewed papers were casuistic reports and few of the included papers were specifically designed to search for malignant cells. Ovarian tissue from 422 patients has been subject to testing for malignant cells by imaging, histology, immunohistochemistry, molecular biology, animal- or clinical transplantation. In 31 (7 %) of the cases the applied test raised suspicion of malignant cell infiltration. No transplantation-related relapse of cancer has been reported after 33 transplantations of frozen/thawed ovarian cortex.
Conclusion The quality and strength of the evidence is generally low and prospective studies are needed. The risk of re-introducing a malignant condition when transplanting ovarian tissue depends on the particular disease. Based on the available data, the risk was estimated: Leukaemia: HIGH. Gastrointestinal cancers: MODERATE. Breast cancer, sarcomas of the bone and connective tissue, gynaecological cancers, Hodgkin's and Non-Hodgkin's Lymphoma: LOW.

Keywords Fertility preservation · Ovarian tissue cryopreservation · Minimal residual disease · Safety · Cancer · Cryobiology · Ovarian reserve · Lymphoma · Breast cancer · Leukaemia

Capsule Cryopreserved ovarian tissue from cancer patients may contain malignant cells capable of causing relapse of the malignancy if the tissue is retransplanted. The literature is reviewed and the estimated risk of reintroduction of the most common malignancies in case of retransplantation of the tissue is proposed.

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Background

For many premenopausal women facing gonadotoxic treatment for malignant disease, preservation of fertility is an important subject. Oocyte and embryo cryopreservation are effective and available techniques but both require a period of hormonal stimulation before a result is obtained.

Ovarian tissue can be harvested and cryopreserved from day-to-day, does not delay the treatment and is the only available method for pre-pubertal girls [61]. To date at least 18 live births are the result of transplantation and of frozen/thawed ovarian cortex [4, 5, 12, 13, 18–20, 22, 51, 60, 66, 68, 76, 77].

In order to save as many of the primordial follicles as possible from the harmful effect of chemotherapy, the ovarian cortex is usually removed before therapy is initiated. Under these circumstances, any malignant cells in the ovarian tissue are not destroyed by the chemotherapy and thus it cannot be excluded that malignant cells from the original malignancy are present and viable when the frozen material is thawed and transplanted. The presence of such cells

therefore presents a potential risk of a generalised or local relapse if the tissue is thawed and transplanted.

Ovarian metastases (OM) have been described for most malignancies: Breast cancer (BC), lung cancer, renal tumors, neublastomas, Ewing's sarcoma, Hodgkin's lymphoma (HL), Non-hodgkins lymphoma (N-HL), biliary duct cancer and other cancers of the gastrointestinal system [34, 36, 39, 44, 82, 83].

In the records of 5,571 female autopsies, 22.4 % had OM from non-gynaecological malignancies [43]. The percentage of OM was highest in cases of gastric carcinoma (55.8 %), followed by colon carcinoma (26.6 %), breast cancer (24.2 %), pulmonary carcinoma (23.4 %), lymphoma (13.3 %), uterine cancer (13.1 %), and leukaemia (8.4 %) [43].

In another retrospective study, most secondary ovarian malignancies (39 %) derived from the gastro-intestinal tract, 28 % from breast cancer and 22 % from endometrial cancer [10].

The relatively high frequency of OM obviously calls for great caution when transplanting the tissue. However, as most of the published data are obtained as results of autopsies, prophylactic or therapeutic oophorectomy or otherwise coincidental, the data do not necessarily reflect the risk of ovarian involvement in patients who are normally offered ovarian tissue cryopreservation. Fertility preservation is usually only offered to patients with a high chance of a long-term survival and these patients will typically have low stage - and limited disease with a minimal risk of dissemination and ovarian involvement. One exception, however, is leukaemia which is a disease of the blood and is hence believed to be present in all tissue.

The aim of this paper is to review the available data describing the safety of transplantation of ovarian tissue from patients who had ovarian cortex cryopreserved prior to treatment for a malignant condition. The review will evaluate laboratory data, animal experiments and most importantly, the clinical experiences with human ovarian tissue transplantation.

Materials and methods

Data search was performed in June 2012. In Embase, the following parameters were used: "cryopreserved ovarian tissue" and "cryopreservation AND fertility AND cancer". In Pubmed, the following parameters were used: "Cryopreservation AND fertility AND ovary", "Cryopreservation AND cancer AND ovary", "cryopreserved ovarian tissue" and MESH: "ovary AND cryopreservation". No language was excluded.

Only peer reviewed, original papers were included. All papers addressing the presence or absence of malignant cells in human ovarian tissues harvested for cryopreservation were included as well as all papers describing human-animal and human-human ovarian transplantation after malignant disease. Based on title and abstract an initial selection performed by the first author (MR) reduced the number of references to 98, all in English. The references were read by all three authors and after further selection, 41 references were included in the review. Additional information was achieved by contacting the author regarding one paper [40].

In order to have consistent and transparent evaluation of the strength of evidence of each included study, a validated scoring system was applied. The GRADE (Grading of Recommendations Assessment, Development and Evaluation) system was developed for evaluation of evidence for clinical guidelines and can readily be used for clinical reviews. In the GRADE system the strength of the evidence and the weight of the recommendations were analysed as listed below:

The quality of evidence (QE) was graded *high, moderate, low* and *very low* quality based on the criteria in Table 1 [31–33].

Each study was evaluated by its design, methods and magnitude. Prospective studies designed to search for malignant cells were rated higher than studies where malignant cell contamination was a secondary result. Similarly, the number of cases in the study and careful consideration of the method applied increased the rating.

In the following, the results are categorized after test and condition. One paper may therefore perform well in one category (i.e. histology) and be rated less in another category (i.e. clinical transplant). Hence, one paper may receive two different ratings of *quality of evidence* depending on the actual category.

Results

Data quality

Totally, 42 studies were included. No randomised or controlled studies were found. Prospective analysis of data was performed in some studies but only on previously gathered material. The majority of the data was obtained from casuistic reports presenting retrospective data.

In 12 papers, detection of malignant cells in cryopreserved ovarian tissue was the primary objective of the study. In the remaining papers, the search for malignant cells was listed as a remark without detailed information of the method.

In 27 papers, only a single case was described and 12 cases were described in more than one paper. In these cases the references were not excluded if each paper provided new information about the case. The cumulative results were included but each case only counted once.

In the following, the results are presented according to diagnosis and the applied method. The findings are summarised in Table 2.

Table 1 Quality of evidence

High	Further research is unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain

Adapted after Huyatt et al, BMJ 2008

Ovarian tissue from 422 patients has been subject to testing for malignant cells by imaging, histology, immunohistochemistry (IHC), molecular biology, animal- or clinical transplantation. In 31 (7 %) of the cases the applied test raised suspicion of malignant cell infiltration.

No relapse of cancer was reported after 33 autotransplantations of frozen/thawed ovarian cortex.

Diagnoses

The diagnoses most commonly described were: Hodgkin's Lymphoma (HL), Non-Hodgkin's Lymphoma (N-HL), Breast Cancer (BC) and Leukaemia. Single, small studies described various sarcomas, gynaecological cancers and cancers of gastrointestinal system.

Hematological malignancies

Leukaemia

In a study by Meirow, ovarian cortical biopsies (3×3 mm) from nine patients with leukaemia were evaluated. (Chronic Myeloid Leukaemia (CML) *N*=3; Acute Myeloid Leukaemia (AML), *N*=5 and Myelodysplastic syndrome, *N*=1) Histology revealed no leukemic infiltration, IHC was not performed [50].

In two of the three patients with CML ovarian tissue was evaluated for residual disease using PCR for the CML-specific Philadelphia chromosome t(9:22) by detection of the BCR-ABL mRNA transcript. In the first patient qualitative RT-PCR for the BCR-ABL mRNA transcript was negative. However, 5 years later a more sensitive quantitative real time RT-PCR (RT-qPCR) of the same tissue fragment was positive and transplantation was not performed. In the second patient, both RT-PCR and RT-qPCR were negative [50]. All though a small number, the study was a comprehensive and prospective case series on previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *low*.

No malignant cells were discovered using histology and IHC [62] when one cortical biopsy from each of 26 patients with leukaemia (ALL *N*=13; CML *N*=5; AML *N*=7, juvenile myelomonocytic leukaemia *N*=1) was examined.

However, known molecular markers from the leukaemia were available for 8 of the 26 patients and quantitative RT-PCR was positive in six of the eight patients: four cases of CML, one case of ALL and one case of AML. The study was a comprehensive and prospective case series on previously gathered material and designed to search for malignant cells. The results were confirmed by positive and negative controls; Quality of evidence (QE): *moderate*.

Dolmans and co-workers [17] examined cortical biopsies (4×2 mm) from six patients with CML and 12 with ALL. Histology was negative for malignant cells in all cases. Among the six patients with CML, two were positive by RT-qPCR. Among the 12 patients with ALL, 10 had known molecular markers. Of the 10 patients, 7 were RT-qPCR positive.

The authors grafted 18 severe combined immunodeficient (SCID)-mice with ovarian tissue from each of the patients with CML (*N*=6) and ALL (*N*=12) for 6 months.

Five of the 12 mice grafted with tissue from patients with ALL developed leukaemia in the grafts, peritoneum or internal organs. None of the mice grafted with ovarian tissue from patients with CML developed macroscopic or microscopic evidence of leukaemia. In three of the five positive cases, pre-transplant ovarian tissue PCR had been positive; in the last two cases no molecular marker was available. None of the mice grafted with PCR-negative ovarian tissue developed leukaemia but conversely, some mice grafted with ALL-PCR positive and CML-PCR positive ovarian tissue did not develop leukaemia.

The study was a comprehensive and prospective case series on previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *moderate*.

In the Study by Greve et al. [30] a similar experiment was performed. Ovarian tissue from 25 women with leukaemia —17 of whom were in complete remission at the time of tissue harvest—was transplanted to mice for 20 weeks. All patients were examined with histology and IHC and in seven patients RTq-PCR was possible. RT-qPCR was positive in four patients—two of whom were in complete remission. After murine transplantation, examination of the grafts revealed no signs of malignant cells neither by histology, IHC or RTq-PCR. The authors conclude that complete remission at the time of tissue cryopreservation may reduce the risk of malignant cell survival in the graft and thereby the risk associated with tissue transplantation. The study was a comprehensive and prospective case series on

Table 2 Diagnoses, method and result of tissue examination for patients undergoing ovarian tissue cryopreservation

Reference	*N*	Imaging	Histology	IHC	PCR	Human-Animal	Clinical
Leukemia							
Meirow et al. [50]	9	÷	÷		☒ (1 of 2)		
Dolmans et al. [17]	18		÷		☒ (9 of16)	☒ (5 of18)	
Rosendahl et al. [62]	26		÷	÷	☒ (6 of 8)		
Rosendahl et al. [64]	7					÷	
Greve et al. [30]	25		÷	÷	☒ (4 of 7)	÷	
Subtotal (positive test)	85	9(0)	78(0)	51(0)	33(20)	50(5)	0
Non-Hodgkin's Lymphoma							
Meirow et al. [47], Meirow et al. [50, 51]	16	☒ (*N*=2)	÷ (*N*=14)		÷ (2 of 14)		÷ (*N*=1)
Kim et al. [41]	5		÷			÷	
Andersen et al. [4], Schmidt et al. [70, 72], Tryde Schmidt et al. [80]	1						÷
Dolmans et al. [16], Donnez et al. [21]	1						÷
Schmidt et al. [72]	1						÷
Akar et al. [2]	1						÷
Rosendahl et al. [64, 65]	2					÷	–
Stern et al. [78]	1		÷				÷
Subtotal (positive test)	28	16(2)	20(0)	0	2(0)	7(0)	6(0)
Hodgkin's Lymphoma							
Meirow et al. [48]	7		÷				
Meirow et al. [50]	33	÷	÷	÷ (*N*=1)			÷ (*N*=1)
Radford et al. [59]	1		÷				÷
Kim et al. [41]	13		÷			÷	
Seshadri et al. [73]	26		÷	÷			
Bittinger et al. [8]	1		☒	☒			
Donnez et al. [18, 21]	1						÷
Rosendahl et al. [63], Schmidt et al. [70, 72]	1						÷
Schmidt et al. [70, 72]	1						÷
Oktay [53], Oktay et al. [56]	1						÷
Demeestere et al. [11–13]	1		÷				÷
Sanchez et al. [67]	3						÷
Dolmans et al. [16], Donnez et al. [21]	1						÷
Andersen et al. [4], Schmidt et al. [72]	1						÷
Silber et al. [75]	1						÷
Schmidt et al. [72]	1						÷
Rosendahl et al. [64, 65]	7					÷	
Dittrich et al. [14]	1						÷
Subtotal (positive test)	101	33(0)	82(1)	28(1)	–	20(0)	15(0)
Breast cancer							
Sanchez-Serrano et al. [69]	69		÷	÷			
Rosendahl et al. [64, 65]	51		÷	÷			
Rosendahl et al. [64, 65]	9					÷	

Table 2 (continued)

Reference	*N*	Imaging	Histology	IHC	PCR	Human-Animal	Clinical
Azem et al. [6]	13		÷	÷			
Kim et al. [40]	1		÷				÷
Sanchez et al. [67], Sanchez-Serrano et al. [68]	1						÷
Oktay et al. [54]	1		÷				÷
Andersen et al. [4], Rosendahl et al. [64, 65], Schmidt et al. [72]	1						Local relapse
Subtotal (positive test)	146		135(0)	133(0)		9(0)	4(0)
Sarcomas							
Andersen et al. [4], Ernst et al. [22], Schmidt et al. [72]	1						÷
Azem et al. [6]	13		÷				
Abir et al. [1]	8		÷ (7 of 8)	÷	+ (1 of 8)		
Rosendahl et al. [64, 65]	5					÷	
Ernst et al. [23]	1						÷
Subtotal (positive test)	28		20(0)	7(0)	8(0)	5(0)	2(0)
Cervical cancer							
Azem et al. [6]	2		÷				
Kim et al. [40]	3		÷				1/3 local relapse
Schmidt et al. [72]	1						÷
Endometrial cancer							
Azem et al. [6]	1		÷				
Ovarian malignancies							
Lotz et al. [46]	23		÷			÷ (*N*=10)	
Gastro-intestinal system							
Azem et al. [6] (colon)	1		÷	÷			
Dittrich et al. [15] (anal)	1						÷
CNS tumours							
Azem et al. [6]	1		÷				
Donnez et al. [20]	1		÷				÷
Total (positive test)	422	58(2)	367(1)	220(1)	43(21)	101(5)	33(0)

N Number of patients; *IHC* Immuno Histochemistry; *PCR* Polymerase Chain Reaction; *Clinical* transplantation of frozen/thawed human ovarian cortex to human host; ÷: Biopsy/transplantation performed. Result negative or not described. ☒: positive test for malignant cells. "(Positive test)": number of cases with a positive test result

previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *moderate*.

Non-Hodgkin's Lymphoma (N-HL)

Prior to harvesting of the ovarian tissue, 16 patients with NH-L were assessed with US and/or CT/Pet-CT. In two patients, imaging raised suspicion of disease in the ovaries and ovarian tissue was not removed [50]. Cortical biopsies (3×3 mm) of the remaining 14 patients were negative for lymphoma cells by histological evaluation.

In two patients PCR was used to search for malignant cells. In both patients PCR was negative, and tissue was transplanted—in one case leading to the birth of a healthy

child [51]. The study was a prospective case series on previously gathered material and designed to search for malignant cells; QE: *low.*

Kim [41] evaluated ovarian cortex (8 mm) from 5 patients with N-HL. Light microscopy showed no evidence of malignant cells. The authors then transplanted the human tissue subcutaneously to non-obese diabetic SCID mice for up to 16 weeks. None of the mice developed lymphoma of human origin and positive controls supported the validity of the method. The study was a prospective case series on previously gathered material and designed to search for malignant cells; QE: *low.*

In a study by Rosendahl, frozen/thawed ovarian cortical biopsies from two premenopausal women with N-HL were transplanted subcutaneously to immunodeficient mice for 4 weeks. Macroscopic evaluation of the grafts and microscopic evaluation after HE staining revealed no signs of reintroduction of disease. However, the tissue was only transplanted for 4 weeks and was not evaluated by a pathologist [64] QE: *very low.*

Preceded by negative histological evaluation in two cases [50, 78], totally six patients with N-HL have received frozen/thawed ovarian transplants [2, 4, 16, 21, 47, 50, 51, 70, 72, 78, 80].

Relapse was not been described. Only in the study by Meirow [50] (QE: *low*) was a thorough evaluation described prior to transplantation. The remaining studies are scored QE: *very low.*

Hodgkin's Lymphoma (HL)

Meirow [48] examined ovarian cortical biopsies from seven patients with HL stage IIB-IV. Histology showed no involvement of HL. In 2008, [50] the same group examined cortical biopsies (3×3 mm) from 33 patients with HL, of whom 11 were considered to be high risk patients (stage IV, infradiaphragmatic disease or pelvic lymph nodes). Histology revealed no malignant cell involvement. Tissue from one patient was further examined with IHC and did not reveal malignant cells. The tissue was later used for autotransplantation with no evidence of relapse within 2 years. Both studies were comprehensive and prospective case series on previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *low.*

Ovarian cortex (8 mm) from 13 patients with HL (3 with primary disease, 10 with recurrent disease) was examined by Kim [41]. Light microscopy showed no malignant cells and the authors transplanted the human tissue subcutaneously to non-obese diabetic SCID mice for up to 16 weeks. None of the mice developed lymphoma of human origin and positive controls supported the validity of the method. The study was a comprehensive and prospective case series on previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *low.*

Examining the ovarian cortex of 26 patients with HL—seven of whom with infradiaphragmatic disease, Seshardi [73] found no evidence of malignant cells using histology and IHC. The study was a comprehensive and prospective case series on previously gathered material and designed to search for malignant cells; Quality of evidence (QE): *low.*

Bittinger and co-workers showed possible ovarian involvement of HL [8]. A 19-year old woman with HL stage IIIB had normal ovarian preoperative ultrasound and macroscopically normal ovaries. Microscopic evaluation and IHC of a single biopsy showed likely ovarian involvement of HL. However, the remaining pieces were thawed and revealed no evidence of HL. The study was based on a single result and needs confirmation. QE: *very low*

In a study by Rosendahl [64] frozen/thawed ovarian cortical biopsies from nine women with HL were transplanted subcutaneously to immune-deficient mice for 4 weeks. Macroscopic and microscopic evaluation of the grafts revealed no signs of reintroduction of disease. However, the tissue was only transplanted for 4 weeks and was not evaluated by a pathologist QE: *very low*

In 15 patients, the frozen/thawed ovarian cortex has been retransplanted [4, 11–14, 16, 18, 21, 50, 53, 56, 59, 63, 67, 70, 72, 75].

A histological evaluation of a biopsy was performed in four cases but the result was only described in three studies [11, 50, 59].

Relapse of HL was not described in any of the cases. All: QE: *very low*

Breast cancer

Azem et al. [6] evaluated two biopsies of ovarian cortex (~5×5–10 mm) from 13 women with breast cancer using histology and IHC. None of the specimens revealed malignant cell involvement. The study was designed for disease detection. However, the stages and histological types of disease were not listed. QE: *very low.*

With histology and IHC Sanchez-Serrano [69] did not find histological evidence of malignant cells in the cortex from six whole ovaries from each of six women and in a total of 100 ovarian cortical biopsies (~7×7 mm) from 63 women with breast cancer stage I-IIIa. The study was designed for disease detection, included a high number of biopsies, was well performed and was clear in its conclusions. QE: *moderate.*

Rosendahl [65] systematically examined one ovarian cortical biopsy (~5×5 mm) from each of 51 patients with breast cancer (malignancy grade I-III) with histology and IHC. The study revealed no evidence of malignant cell infiltration..

The study was designed for disease detection, included a relatively high number of biopsies, was well performed and a positive control confirmed the method. QE: *moderate.*

In another study, Rosendahl described subcutaneous transplantation of ovarian cortex from patients with breast cancer to immunodeficient mice for 4 weeks. Macroscopic evaluation of the grafts and microscopic evaluation after HE staining revealed no signs of reintroduction of disease. However, the tissue was only transplanted for 4 weeks and was not evaluated by a pathologist [64]. QE: *low.*

Kim and colleagues [40] found no malignant cells in a single ovarian biopsy from a woman with a stage IIa ductal adenocarcinoma of the breast. The tissue was used for autotransplantation with no evidence of relapse. The study was not designed for disease detection and presented a single case. QE: very *low.*

Totally, four patients with breast cancer have had re-transplantation of the ovarian tissue. [4, 40, 54, 64, 67, 72]. Only one case documented pre-transplantation histology [40]. None of the cases describe ovarian metastases but one patient developed a local relapse at the site of the original breast tumour approximately 1 year after autotransplantation of ovarian cortex. There was no sign of metastatic activity in the ovarian graft [64]. QE: very *low.*

Sarcomas of the bone and connective tissue

Azem [6] examined two biopsies of ovarian cortex (~5×5–10 mm) from 13 patients with '*sarcoma of the bone—including Ewing sarcoma*' after HE staining and found no malignant cell involvement. The study was designed for disease detection. However, the stages and histological types of disease were not listed. QE: *very low.*

Abir [1] evaluated ovarian cortex from seven of eight patients with Ewing Sarcoma (ES). One biopsy (2×2 mm^2) per patients was evaluated with histology and IHC. None of the biopsies indicated malignant cell involvement. Biopsies from all eight patients were subjected to RT-PCR. In one case, a 14-year old girl, RT-PCR for the *Ewing Sarcoma Friend Leukemia Virus Integration Site I fusion gene* was positive, suggesting ovarian involvement of the sarcoma. The study was designed for disease detection, the material and methods well described and a highly sensitive method was applied. However, the result requires confirmation and the study only comprised eight patients. QE: *low.*

Rosendahl described subcutaneous transplantation of ovarian cortex from patients with breast cancer to immunodeficient mice for 4 weeks. Macroscopic evaluation of the grafts and microscopic evaluation after HE staining revealed no signs of reintroduction of disease. However, the aim of the study was to evaluate follicle survival—not disease transmission [64]. QE: *very low.*

Clinical transplantation to a patient treated for sarcoma has only been described in two cases. The first patient, treated for Ewing Sarcoma, gave birth to three children following autotransplantation and relapse has not occurred 7 years after transplantation [4, 5, 22, 72]; QE: *very low.* Another patient treated for Ewing sarcoma at the time of tissue retrieval was transplanted to induce puberty and relapse was not reported more than 3 years after transplantation [23]; QE: *very low.*

Gynaecological cancer

Cervical cancer

Azem found no histological evidence of malignant cells in two biopsies from each of two patients with cervical cancer [6]. The stage of disease not indicated and the number very low. QE: *very low.*

Kim [40] histologically examined specimens from three patients with stage IIb, IIb and Ib and found no ovarian metastases. All three patients received re-transplantations. One patient with stage IIb disease later experienced a local relapse. As the transplant was in the rectus abdominus muscle, the authors interpret the relapse as coincidental and unrelated to the transplant. The study was not designed for detection of metastases and presents a limited number of cases. QE: *very low*

Schmidt and colleagues [72] performed a clinical re-transplantation. There was no mention of pre-transplant histology or signs of disease relapse in the paper. QE: *very low.*

Endometrial cancer

Evaluation of one biopsy of Azem [6] cortex from one patient with endometrial carcinoma with HE staining showed no evidence of malignant cells. There was no mention of the grade or stage of disease. QE: *very low.*

Ovarian cancer

Lotz et al. [46] examined ovarian cortex from 23 premenopausal patients with epithelial and non-epithelial ovarian malignancies. Light microscopy showed no evidence of malignant cell involvement. Tissue from ten of the patients was transplanted to SCID mice for 24 week. Macroscopic evaluation of the animals as well as histology and IHC revealed no malignant cells. The study was specifically designed for detection of malignant disease and included a relatively large number of patients. However, many different diagnoses were included and generalization is difficult. QE: *low*

Gastro-intestinal cancers

Two studies evaluated the presence of malignant cells in patients with gastro-intestinal cancers. Azem [6] examined

one piece of cortex from a patient with colon cancer, unknown stage of disease, with histology and IHC with no evidence of malignant cells. The study was designed for disease detection, however, the stage and histological type of disease was not listed. QE: *very low.*

Dittrich and colleagues [15] transplanted ovarian tissue to a patient treated for anal cancer. The stage of disease is not listed and, pre-transplantation histology is not described. The authors do not mention relapse of the cancer. QE: *very low.*

CNS tumours

Azem [6] examined ovarian cortex from a single patient with a medulloblastoma of the brain with histology. No malignant cells were discovered. QE: *very low.*

Donnez and colleagues described a patient with a neuroectodermal tumour in the orbita and metastases to the chest who had ovarian tissue transplanted. Pre-transplant evaluation of the tissue revealed no malignant cells and the authors do not mention relapse of disease [20]. The study was not designed for disease detection and presents a single case. QE: *very low.*

Risk of transmission of disease per piece of transplanted cortex

As a single biopsy represents only a small fraction of the ovarian cortex, most transplantations are done with a number of cortical fragments which therefore represent a larger and randomised selection of biopsies. In order to evaluate the risk of disease transmission *per piece* of transplanted cortex, the information was extracted—where possible—from the publication. The results are listed in Table 3, Some authors list the material in mm^2, some in number of biopsies. For comparison we have uniformed the result to numbers of biopsies measuring 5×5 mm (25 mm^2). Where the size of the biopsies was not listed, 5×5 mm biopsies are assumed.

Totally, at least 320 5×5 mm biopsies have been transplanted with no report of relapse.

Discussion

Data quality

This review demonstrates that there is a lack of systematic studies evaluating the risk of malignant cell infiltration in ovarian tissue from patients with malignant disease who undergo OTC for fertility preservation. Each study was scored based on the validated GRADE system where the quality of evidence (QE) was rated *high, moderate, low* and *very low* quality based on the criteria in Table 1.

Only five studies were rated *moderate,* 11 rated *low* and the remaining studies were rated *very low*. This result probably reflects that ovarian tissue cryopreservation and –transplantation is a relatively new procedure and the ethical obstacles involved in gathering human material from control patients. Furthermore, designing studies where most biopsies must be assumed to be disease-free is difficult as only a true positive result can confirm the method. Finally, the optimal procedure for detecting residual malignant cells in cryopreserved ovarian tissue is not clear and furthermore, the method is likely to vary with the malignancy in question.

In addition to a lack of studies, publication bias may result in lack—or delay—of publication of cases where malignant cells have indeed been found in tissue that, as a consequence, was not transplanted.

Detection of malignant cells in cryopreserved ovarian tissue

In this review, investigators had used preoperative imaging, histology and immunohistochemistry, and PCR of ovarian tissue to search for malignant cells. Some authors had transplanted the ovarian tissue to animals in order to evaluate the potential of malignant cells to nest and reproduce in the host animal. Finally clinical re-transplantations were performed with and without pre-transplant histology.

Preoperative imaging may be helpful in detecting with large ovarian masses and is proposed as a screening. However it is unable to detect smaller lesions that may contain malignant cells.

Histology and IHC are sensitive methods and can detect clusters of malignant cells but may not detect single cells that may in fact, if transplanted, cause relapse of disease. Furthermore, arbitrarily, the examined tissue fragment is destroyed in the procedure and cannot be transplanted and since the examined biopsy merely represents a small fragment of the total ovary it does not exclude malignant cells in another fragment of cortex.

RT-PCR is a highly sensitive method to detect small quantities of genetic material but requires a specific and known sequence in the examined material in order to be useful. Further, a positive RT-PCR result only confirms the presence of the malignant cells—but cannot reveal the viability or malignant potential of the cells if transplanted. Finally, it is unclear how many malignant cells are actually required to cause relapse of the disease and the number is likely to vary between individuals and malignancies.

Human transplantations are obviously very informative, but are not without risk to the patient and until a higher number of transplantations have been performed, they only represent individual cases and cannot statistically predict the risk of re-introduction of the malignancy.

Table 3 Estimated number of pieces of ovarian cortex (5×5 mm) transplanted

Reference	Original number / area mm^2	Uniformed number of pieces 5×5 mm (25 m^2)
Non-Hodgkin's Lymphoma		
Meirow et al. [47, 50, 51]	4 (5 mm)	4
Andersen et al. [4], Schmidt et al. [70, 72], Tryde Schmidt et al. [80]	18 (5×5 mm)	18
Dolmans et al. [16], Donnez et al. [21]	200 mm^2	8
Schmidt et al. [72]	20 (5×5 mm)	20
Akar et al. [2]	12 (7×5 mm)	17
Stern et al. [78]	88 mm^2	4
Subtotal		71
Hodgkin's Lymphoma		
Meirow et al. [50]	?	?
Radford et al. [59]	2 (1×0.5 mm)	< 1
Donnez et al. [18, 21]	? (12×5 mm)	> 2
Rosendahl et al. [63], Schmidt et al. [70, 72]	20 (5×5 mm)	20
Schmidt et al. [70, 72]	18 (5×5 mm)	18
Oktay [53], Oktay et al. [56]	?	?
Demeestere et al. [11–13]	18 (5×5×2)	18
Sanchez et al. [67]	2 (15×20 mm) per patient	72
Dolmans et al. [16], Donnez et al. [21]	5 (5×10 m)	10
Andersen et al. [4], Schmidt et al. [72]	22(5×5 mm)	22
Silber et al. [75]	?	?
Dittrich et al. [14]	6 (1×2 mm)	0.5
Subtotal		> 145,5
Breast cancer		
Kim et al. [40]	8–10 (btw 5×5–5×10 mm)	~15
Sanchez et al. [67], Sanchez-Serrano et al. [68]	2 (15×20 mm)	24
Oktay et al. [54]	15 (btw 5×5×1–15×5×2)	~25
Andersen et al. [4], Rosendahl et al. [64, 65], Schmidt et al. [72]	19 (5×5)	19
Subtotal		~63
Sarcomas		
Andersen et al. [4], Ernst et al. [22], Schmidt et al. [72]	7 (5×5)	7
Ernst et al. [23]	2 (4×5)	~2
Cervical cancer		
Kim et al. [40]	8–10 (btw 5×5–5×10 mm)	~15
Schmidt et al. [72]	16 (5×5 mm)	16
Gastro-intestinal system		
Dittrich et al. [15] (anal)	6 (1×2 mm) and additional fragments to the pelvic wall	>0.5
CNS tumours		
Donnez et al. [19, 20]	?	?
Total		>320 pieces (5×5 mm)

Taking these limitations into account, it seems that xenotransplantation experiments could be a valuable method to evaluate the risk of disease transmission. Representative biopsies of ovarian cortex from patients with various diagnoses undergoing OTC can be systematically transplanted to immune incompetent host animals such as mice which then act as bioincubators to propagate potential malignant cells. The transplants and the animals can, after a suitable period of incubation, be evaluated them for possible appearance of disease.

Even though additional studies are desirable, guidelines can only be based on current knowledge. Based on the reviewed studies general guidelines are outlined below. These suggestions are summarised in Table 4.

Leukaemia

Leukaemia is a disease of the blood and is therefore presumed to be present in all organs. No human transplantation has so far been described to a patient who was treated for leukaemia due to a theoretical risk of introduction of malignant cells. Four studies [17, 30, 50, 62] have now firmly supported a restrictive approach towards transplantation in case of previous leukaemia. PCR-positive genetic material—consistent with malignant cells of the particular disease—has been found in the ovarian tissue from patients with CML and AML and ALL. One study showed that when transplanted with ovarian cortex from patients with ALL, mice developed leukaemia in the grafts, peritoneum or internal organs [17]. As a comforting result, none of the mice transplanted with PCR-negative ovarian tissue developed the malignancy and being in complete remission at the time of tissue harvest may reduce the risk of malignant cell infiltration. However, the numbers studied were too few to generalize.

Taken together, transplantation of ovarian cortex from patients with leukaemia is likely to reintroduce or boost the malignancy and should be avoided at this time. However, the type of disease and whether or not the patient is in complete remission at the time of tissue harvest may influence on the risk of disease transmission. Based on the presented studies however, there is not enough evidence to back such a theory.

Cryopreservation of ovarian tissue can however, still be offered to this group as other means of fertility preservation are emerging. Depending on the age of the patient, the ovarian cortex and medulla contain hundreds to ten-thousands primordial follicles that may be isolated [42]. It is the hope that in near future, isolated primordial follicles, which are free of somatic cells and hence disease-free, can be matured in vitro or transplanted to the remaining native menopausal ovary and produce viable MII oocytes [79].

Table 4 Best estimate of risk of the transmission of malignant disease

	Overall quality of evidence	Strength of recommendation	Risk of transmission
Leukemia	Moderate	Strong	High
Hodgkin's Lymphoma	Moderate	Strong	Low
Non- Hodgkin's Lymphoma	Low	Weak	Low
Breast cancer	Moderate	Strong	Low
Sarcomas	Low	Weak	Low
Gastro-intestinal cancer	Low	Weak	Moderate
Gynecological cancer	Low	Weak	Low

Lymphomas

Survival rates in lymphoma patients have increased substantially over the past four decades [38]. Treatment for N-HL and advanced stage HL [24], include alkylating agents and thereby an increased risk of chemotherapy-induced infertility [49] and fertility preservation should be considered in these patients.

However, there is a small potential risk of ovarian involvement in lymphoma disease. Totally, the ovarian cortex of 82 patients undergoing OTC for HL has been histologically examined and only in one case [8], were malignant cells suspected. Further, ovarian cortex of 20 patients with HL has been xenotransplanted without evidence of disease in the host indicating, but not confirming, that negative histology is associated with a low risk of disease transmission. Finally, 15 patients with HL, some in stage IV, have received transplantations of ovarian cortex without reports of relapse.

For N-HL, histology, IHC and molecular biology studies have been negative and six patients with N-HL, including some with advanced disease, have received transplantations without experiencing relapse.

Overall, based on the available knowledge, autotransplantation of ovarian cortex from patients with HL and N-HL appears safe, provided that pre-transplant evaluation of the tissue is negative.

Breast cancer is one of the leading causes of cancer in fertile age. Breast cancer can metastasize to the ovaries, most often in the case of lobular infiltrating carcinoma and predominantly results in bilateral, asymptomatic lesions correlated to a 3-year median survival [7, 57]. Nevertheless, development of an ovarian tumour after diagnosis with BC is more likely to be of primary ovarian origin than a BC-metastasis [7]. In spite of the fact that BC may metastasize to the ovaries, ovarian cortex from 135 patients has been examined histologically without evidence of malignant cell involvement. Further, transplantation of ovarian cortex has been performed to four patients with BC without evidence of relapse in—or caused by—the graft. Overall, this data is reassuring and indicates a very low risk of malignant cell transmission.

Sarcomas of the bone and connective tissue

Based on the presented studies, sarcomas may metastasize to the ovaries. However, detection of potentially malignant cells by PCR does not necessarily mean that the sarcoma will be reintroduced if the tissue is transplanted. Two patients treated for sarcoma has received transplantation of ovarian tissue and no relapse was reported 7 and 3 years after transplantation respectively [4, 23]. Further studies, such as xenotransplantation studies are however required to further address the risk. Presently, however, based on the presented cases, the risk of malignant cell transmission is considered to be low.

Cervical carcinoma

Ovarian cortex of five patients with cervical carcinoma was without malignant cells and human transplantation with frozen thawed tissue has been performed in four patients. In one case, local relapse occurred. This is to be expected in the natural course of the disease and does not indicate a negative role of the ovarian graft. The material is obviously insufficient to determine the risk of ovarian involvement in OTC-patients, but ovarian metastases from uterine cervical carcinoma are not exceptional. In 3,471 cases of cancer of the uterine cervix stage stage Ib1- IIb, ovarian metastases occurred in 5.31 % and 0.79 % cases of adenocarcinoma and squamous cell carcinoma respectively [74]. Although most cervical cancers are of squamous origin, these data show the importance of caution when transplanting cryopreserved tissue to patients after treatment for carcinoma of the uterine cervix and the risk of disease transmission is considered *medium*.

Only few cases were reported for CNS-, gastro-intestinal, ovarian- and endometrial cancers. These groups comprise various diagnoses and clear recommendations that apply for all are difficult to make. Tumours from the gastro-intestinal tract have, however, represented the majority of ovarian metastases in retrospective analyses of autopsies [10, 43] and based on the high affinity for the ovaries, the risk of re-introducing malignant cells must be considered *medium* when transplanting ovarian tissue from a patient with a GI-tumour. Again, each case has to be viewed individually with respect to tumour biology, type and stage, pre-transplant histology and immunohistochemistry and if possible xenotransplantation-studies.

Treatment with chemotherapy before or after removal of ovarian tissue

It is not unlikely that patients treated with chemotherapy before the ovarian tissue is removed may have a lower risk of harbouring malignant cells in the cryopreserved material. This may in particular be the case for patients with leukaemia who sometimes undergo stem cell transplantation in a state of complete remission [17, 30]. However, in the analysed material, the data was insufficient to confirm this theory.

Transplantations of ovarian tissue to immunodeficient mice to detect malignant cells has proven to a good model [17] but the efficacy depends on the specific cancer of the patients, the mouse strain and sex, the transplantation site and the observation period.

The ultimate limitation of the observation period is the life span of a mouse. Transplantation of surgical specimens of malignant material has shown that most tumors that will grow on nude mice grow before 20 weeks and waiting longer only reveals few cases [25, 45]. When looking for malignant cells in ovarian tissue the cells are of metastatic origin and these cells grow faster on mice [25]. The rate with which malignant human tumors grow on mice varies and depends on the cancer type [27, 37, 58] as well as on the strain of the mouse [52]. Further, when choosing the graft site both optimal conditions for the malignant cells and the ovarian cortex have to be considered. The ovarian cortex acts as a carrier and provider of vascularisation and nutrition to the malignant cells and thus it is of great importance how the ovarian cortex responds to the transplantation. Different graft sites have been used with success: Under the kidney capsule [28, 29, 55], intraperitoneally [3, 81], the back muscle [26], the ovarian bursa [9] and subcutaneously [71]. One study compared subcutaneous with intraperitoneal transplantation and found a higher take rate of human hematopoietic cells lines when grafted subcutaneously [35].

In conclusion, transplantation of frozen/thawed ovarian tissue may potentially re-introduce the malignancy. For most conditions, however, the risk is very low and is presumably related to the stage of disease at the time of OTC. Based on the available information, general guidelines have been made in this review for the most common conditions leading to OTC (Table 4).

Any transplantation of frozen/thawed ovarian cortex to a patient with a previous malignancy should always be preceded by extensive information to the patient, examination of a representative biopsy by histology, IHC and if possible molecular biology. The gold standard, however, must be considered transplantation of a representative biopsy to a suitable host animal for a period of 20 weeks.

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