Assessing Safety in Ovarian Tissue Transplantation

Marie-Madeleine Dolmans
and Rossella Masciangelo

Introduction

Thanks to marked improvements in cancer treatments over recent years, there is a growing population of young women whose fertility potential is impaired as a result of lifesaving gonadotoxic therapy, causing premature ovarian insufficiency. As statistics continue to rise, it is vital that we give these women a chance to preserve their fertility. However, patients who require immediate oncological treatment and indeed prepubertal girls are not in a position to undergo ovarian stimulation to produce oocytes for freezing. Hence, their only option is cryopreservation of ovarian tissue prior to treatment for future reimplantation. Fragments of tissue can be swiftly and easily retrieved, frozen, and stored until the patient is declared free of disease, before being grafted back to the pelvic cavity when she is ready to conceive [1]. This procedure restores ovarian function over 95% of cases, and it is often maintained for 5–7 years. The younger the patient at the time of biopsy, the larger the ovarian reserve, so the higher the chances of conception. To date, over 130 live births have been obtained using this approach.

Unfortunately, not all cancers are the same in terms of risk to patients, and there is great concern about the possible presence of malignant cells in frozen ovarian tissue. It is essential that we rule out any potential tissue contamination to avoid reimplanting malignant cells that could cause recurrence of the primary disease. To do so, we must identify and examine the risks posed by different cancers but also investigate ways of eliminating cancer cells from frozen-thawed tissue in case of high-risk malignancies. In this chapter, we will explore the different indications for ovarian tissue cryopreservation and reimplantation and assess the risk involved according to cancer type: high risk, moderate risk, and low risk (Table 1).

Hematological Malignancies

Leukemia

Leukemia is one of the most common cancers in children and adolescents [3], and also one of the most curable, with survival rates exceeding 80%. However, it requires prompt and aggressive

M.-M. Dolmans (✉)
Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

Department of Gynecology, Cliniques Universitaires Saint Luc, Université Catholique de Louvain, Brussels, Belgium
e-mail: marie-madeleine.dolmans@uclouvain.be

R. Masciangelo
Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

M. Grynberg, P. Patrizio (eds.), *Female and Male Fertility Preservation*,
https://doi.org/10.1007/978-3-030-47767-7_13

Table 1 Risk of ovarian metastasis according to cancer type

High risk (>10%)	Moderate risk (2–10%)	Low risk (0–2%)
Leukemia	Breast cancer, advanced stage	Breast cancer, early stage
Neuroblastoma	Colorectal cancer	Squamous cell carcinoma of the cervix
Burkitt lymphoma	Adenocarcinoma of the cervix	Hodgkin lymphoma
	Non-Hodgkin lymphoma	Rhabdomyosarcoma
	Ewing sarcoma	Soft tissue sarcoma
	Ovarian cancer	
	Borderline ovarian tumor	

From *Dolmans and Masciangelo, Minerva gynecologica, [2]*

chemotherapy with alkylating agents and often leaves patients infertile, making them prime candidates for ovarian tissue cryopreservation. Because leukemia is blood-borne, malignant cells reside in the bloodstream, so the threat of ovarian involvement is very real when tissue is reimplanted. It is therefore vital to discern any minimal disseminated disease prior to grafting.

Histology and immunohistochemistry usually constitute the first port of call but may fail to detect malignant cells in frozen-thawed ovarian tissue specimens. Polymerase chain reaction (PCR) analysis yields a more accurate picture. Indeed, the BCR-ABL gene was detected by RT-qPCR in the ovarian tissue of a chronic myeloid leukemia (CML) patient, thereby avoiding potentially unsafe transplantation of the tissue back to this subject [4].

Our team [5] also investigated the presence of leukemic cells in ovarian tissue frozen during the active phase of the disease, both from CML and acute lymphoblastic leukemia (ALL) patients, first by PCR and then by xenografting to immunodeficient mice. PCR revealed contaminated ovarian tissue issuing from 70% of ALL patients and 33% of CML patients. After xenografting the tissue, none of the CML-grafted mice developed the disease, while almost 50% of those grafted with ALL tissue were invaded (Fig. 1).

Rosendahl and Greve analyzed ovarian tissue from leukemia patients in complete remission [6, 7]. RT-qPCR identified malignant cells in ovarian tissue in two out of four patients with known molecular markers, but these results were not confirmed by subsequent xenografting, as no leukemic cells were detected by RT-qPCR after 20 weeks of grafting [6]. It therefore appears that ovarian tissue from leukemia patients in complete remission does not contain viable malignant cells able to transmit the disease, but specimens taken during the active disease phase should not be used for transplantation.

However, this does not mean that those at risk of ovarian metastasis should give up hope of ever giving birth. Indeed, there are two existing options for these leukemia patients to use their cryopreserved ovarian tissue. The most straightforward is to check very carefully for the absence of malignant cells in a particular patient. If all the safety tests prove negative, a multidisciplinary decision may be taken, along with informed patient consent, to cautiously graft some fragments back. This approach was adopted by the Israeli team, who reported the first live birth after tissue reimplantation in an acute myeloid leukemia patient [8]. In this instance, the tissue was frozen when the patient was in complete remission and prior to hematopoietic stem cell transplantation. Three fragments of frozen-thawed ovarian tissue were analyzed by light microscopy, cytogenetic analysis, next-generation sequencing, and xenotransplantation, and none were found to contain leukemic cells. Transplantation was then performed, allowing the patient to conceive twice, once after IVF and once spontaneously.

If the safety tests reveal a risk, the second option is isolating and purging individual follicles that can subsequently be grafted back to patients inside a scaffold (artificial ovary) [9]. To this end, ovarian tissue fragments undergo a process of enzymatic digestion, and isolated follicles are effectively washed several times [10]. In a previous study, suspensions of these follicles,

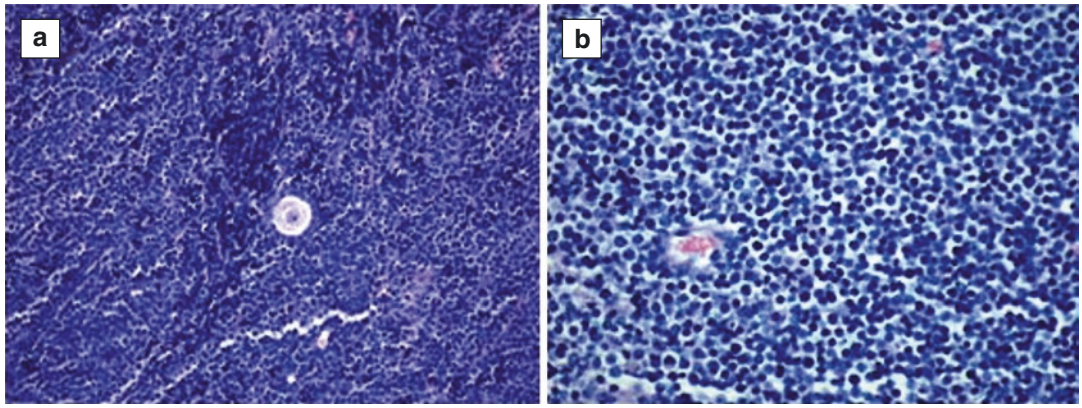

Fig. 1 (**a**) Ovarian follicle surrounded exclusively by lymphocytes; normal ovarian stroma is no longer present. Magnification ×100. (**b**) Massive lymphocytic invasion in an ovarian xenograft. Histological abnormalities were identified as malignant and attributed to leukemic invasion. Magnification ×200. Adapted from *Dolmans, Blood, [5]*

enzymatically digested from the ovarian tissue of 12 leukemia patients, did not show the presence of disease [11]. We must nevertheless tread very carefully; autotransplantation of ovarian tissue should be discouraged in leukemia patients if there is any sign of risk, but grafting of isolated follicles inside a specially created matrix may well prove feasible in the future.

Lymphoma

Hodgkin's (HL) and non-Hodgkin's lymphoma (NHL) are also frequent indications for ovarian tissue cryopreservation and transplantation, but while HL is in the low-risk category, NHL appears to carry a greater risk (moderate risk). A number of teams have attempted to evaluate the safety of autotransplantation in HL patients, and none have detected any malignant cells in their cryopreserved ovarian tissue, even in patients with advanced stage disease [4, 12–16], with the exception of one case report that identified malignant cells in the ovary of a patient with stage III HL [17]. However, there are no reports in the literature on HL recurrence after grafting, so we can conclude that the overall risk of ovarian metastases in case of HL may be considered low and autotransplantation safe.

Concerning NHL, no ovarian involvement was found by histology in the few studies conducted [13, 4]. Kim's group performed xenografting experiments with ovarian tissue from five NHL patients and was unable to prove disease recurrence [13], but Meirow reported one case of a solid pelvic mass in a 33-year-old patient affected by high-grade B-cell NHL, in whom cryopreservation was not carried out [4]. Dolmans et al. also detected malignant cells in the cryopreserved ovarian tissue of two NHL patients by histology and anti-CD20 immunohistochemistry [12] (Fig. 2). Hence, caution is required in case of NHL, especially when patients present in the leukemic phase of the disease, often associated with cystic ovarian masses [4].

Gynecological Malignancies

Breast Cancer

Breast cancer is the most commonly encountered malignancy in women [18]. Although ovarian metastases were found in almost 25% of breast cancer patients at autopsy evaluation [19], two teams were unable to locate any malignant cells in the cryopreserved ovarian tissue of breast cancer patients by immunohistochemical detection methods [20, 21].

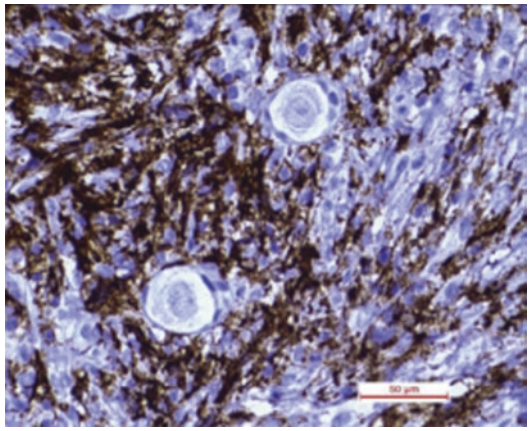

Fig. 2 Ovarian fragments from a non-Hodgkin's lymphoma (NHL) patient. Massive invasion by NHL cells in the cortex is shown by anti-CD20 immunohistochemistry. Adapted from *Dolmans, F&S, [12]*

Nevertheless, the suitability of breast cancer patients for frozen-thawed ovarian tissue transplantation depends on the stage of the primary disease. Reimplantation appears to be safe in women with low-stage breast cancer [22], while there is a risk in case of advanced stage breast cancer [23]. In a previous study by our group [23], qPCR detected mammaglobin 2 (MGB2) gene expression in 4 frozen-thawed ovarian tissue fragments and 1 graft out of 13 patients, even though histology and immunohistochemistry for epithelial membrane antigen (EMA), HER-2/neu, and gross cystic disease fluid protein-15 (GCD-FP15), as well as xenografting to immunodeficient mice, revealed no malignant cell contamination. These results confirm the potential risk of finding malignant cells in cryopreserved ovarian tissue in advanced stage breast cancer patients [23]. However, it is noteworthy that expression levels of specific breast cancer genes in the ovary are highly variable [24]. Mammaglobin 1 (MGB1) and GCD-FP15 have good positive predictive value to identify breast cancer cells in the ovarian cortex, while MGB2 detection shows greater specificity in the ovarian medulla. In the same study, MGB1 was detected in three out of five ovarian cortex biopsies from early-stage breast cancer subjects, but none with advanced stage disease, and none of the mice grafted with ovarian tissue expressing the marker showed any cancer recurrence. These authors therefore concluded that systematic analysis of the tissue should be encouraged using both immunohistochemistry and molecular tools prior to grafting, irrespective of disease stage.

Ovarian Cancer

Ovarian cancer is fifth on the list in terms of female mortality, and, while most cases occur in postmenopausal women, more than 10% of diagnoses are made in patients of childbearing age [25]. Cryopreservation of ovarian tissue from the unaffected contralateral ovary can be proposed to these patients, but safety concerns are well founded. Indeed, ovarian tumors originate from the same organ that will be cryopreserved and reimplanted, increasing the chances of preserving malignant cells. Grafting tissue back to ovarian cancer patients may therefore be somewhat problematic, as there is always a risk of reintroducing the primary disease along with the transplanted tissue.

Four cases of cryopreservation and subsequent reimplantation have been reported in ovarian cancer patients in the literature [26–29]. Pregnancies and live births were obtained in three patients after grafting, while in the fourth, hormone activity never resumed. In one case, a relapse occurred; the patient had previously had a granulosa cell tumor for which she underwent oophorectomy and prophylactic removal of the contralateral ovary, followed by ovarian tissue cryopreservation. Nine years later, her ovarian tissue was reimplanted, and, after low-dose hormone stimulation and IVF, two embryos were obtained and transferred, leading to the birth of healthy twins. However, during elective cesarean section, tumor dissemination was detected in diaphragmatic and peritoneal tissue, but not in the graft sites [29]. This relapse either could have been directly related to the transplanted ovarian tissue or could have been the consequence of microscopic peritoneal disease that responded to the pregnancy-induced hormone environment.

Minimal disseminated disease in ovarian cancer patients was investigated by Lotz's team by xenografting ovarian tissue from ten patients with ovarian tumors for 24 weeks and then performing histology and pan-cytokeratin analysis by immunohistochemistry [30]. These authors failed to detect any malignant cell contamination. In Kristensen's report, a 23-year-old patient diagnosed with stage 1C ovarian mucinous cystadenocarcinoma underwent tissue cryopreservation for fertility preservation and subsequent heterotopic transplantation to restore fertility 9 years later. After achieving a successful twin pregnancy by IVF, the grafted tissue was laparoscopically removed for safety reasons and refrozen. Histological evaluation revealed that a small number of growing follicles ($n = 5$) were indeed able to maintain normal hormone production and support ovarian function. This group also suggested that removing and refreezing grafted ovarian tissue could be a new way of managing cancer patients at risk of malignant cell recurrence [27].

Borderline Ovarian Tumors

Among ovarian tumors, borderline ovarian tumors (BOTs) account for 10–20% of cases. They are characterized by low malignant potential and mostly affect women of reproductive age, making them optimal candidates for ovarian tissue cryopreservation and transplantation. Masciangelo et al. evaluated the safety of reimplantation of cryopreserved ovarian tissue from 11 BOT patients [31]. Hematoxylin and eosin staining, immunohistochemistry for mucin1 (MUC1) and cytokeratin 7 (CK7), RT-qPCR for the CK7 gene, and xenografting were performed in order to investigate the presence of BOT cells in ovarian tissue. BOT cells were detected in the cryopreserved ovarian tissue and graft of 1 out of 11 patients (9.1%), proving their ability to survive after transplantation (Fig. 3a–f). For this reason, it is essential to conduct preimplantation analysis of cryopreserved ovarian tissue from BOT patients prior to its transplantation, as the risk of reintroducing BOT cells able to survive after grafting exists. It is not possible to exclude development of new BOT cells from grafted ovarian tissue, so patients affected by ovarian cancer or BOTs who wish to conceive by ovarian tissue transplantation could undergo laparoscopic removal of the grafted tissue after pregnancy.

Cervical Carcinoma

Reports on ovarian tissue transplantation after cervical carcinoma have been published by the team of Kim [32–34]. They autografted ovarian tissue from three patients with cervical carcinoma and encountered one pelvic relapse [34].

It is noteworthy that cervical cancer treatment might also involve hysterectomy, so patients requiring hysterectomy must be made aware that restoration of fertility through transplantation is not sufficient to achieve a pregnancy and that surrogacy will be needed. It is also worth mentioning that no pregnancy has ever been achieved after ovarian tissue reimplantation in patients diagnosed with cervical cancer, as confirmed in the latest European series [35].

Bone and Soft Tissue Sarcoma

Sarcomas are a heterogeneous group of tumors that develop from connective tissue. They represent more than 20% of solid tumors in the pediatric population and are the third most common cancer after leukemia and central nervous system tumors. More than 50 types of sarcoma have been identified, but the most frequent forms among infants are rhabdomyosarcoma (RMS) and Ewing sarcoma (EWS). Both RMS and EWS are often diagnosed when already disseminated (20% and 40%, respectively), involving the lungs, lymph nodes, and bone marrow. Standard therapies include surgery, chemotherapy, and radiotherapy, yielding high survival rates but impairing ovarian function and future fertility. Ovarian involvement has been reported in certain types of sarcoma, like EWS and osteosarcoma, but it is rare in RMS [36].

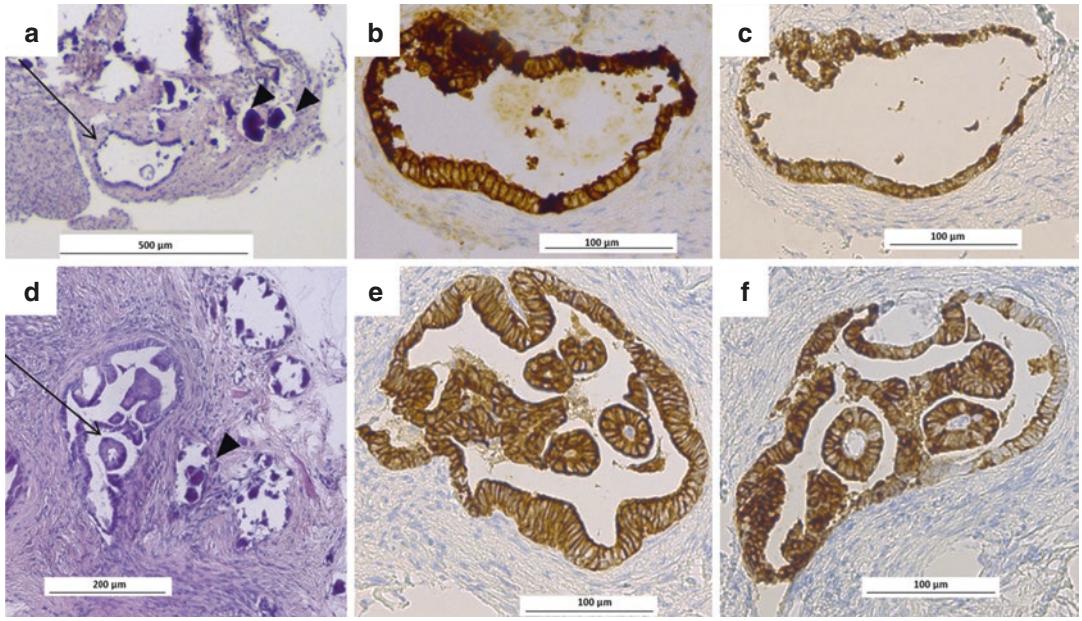

Fig. 3 Frozen-thawed human ovarian tissue and a human ovarian tissue xenograft from one BOT patient after 5 months of grafting to an immunodeficient mouse. (**a**) Histological aspect of frozen-thawed ovarian tissue: a BOT lesion can be seen (black arrow) surrounded by several psammoma bodies (arrowheads). (**b**) Positive immunohistochemical results for CK7 in ovarian tissue. (**c**) Positive immunohistochemical results for MUC1 in ovarian tissue. (**d**) Histology of the xenograft: a BOT lesion can be seen containing papillary projections (black arrow) and surrounded by psammoma bodies (arrowhead). (**e**) Positive immunohistochemical results for CK7. (**f**) Positive immunohistochemical results for MUC1. From Masciangelo et al., Human Reproduction [31]

Three teams have investigated the presence of malignant cells in cryopreserved ovarian tissue from sarcoma patients. Abir analyzed frozen-thawed ovarian tissue from eight EWS patients using anti-CD99 immunohistochemistry and, in five cases, RT-qPCR for the EWS-FLI1 fusion gene. Immunohistochemistry failed to detect any malignant cells, but the EWS-FLI1 fusion gene was identified in one patient, confirming the presence of malignant cells [37]. Greve's team evaluated cryopreserved ovarian tissue from 16 patients (9 EWS, 4 osteosarcomas, 2 synovial sarcomas, 1 chondrosarcoma) by xenografting, histology, and RT-PCR for the EWS-FLI1 fusion gene. They were unable to find any sign of malignant cell contamination in the ovarian tissue, and transplantation to immunodeficient mice did not induce disease recurrence [6]. Finally, Dolmans analyzed cryopreserved ovarian tissue from 26 sarcoma patients (14 EWS, 12 soft tissue sarcomas) after performing genetic characterization of the primary tumor by immunohistochemistry, fluorescence in situ hybridization, and RT-qPCR in order to identify tumoral markers to be tested on the tissue. None of the samples revealed the presence of any malignant cells. Reimplantation in sarcoma patients can therefore be considered safe, with the exception of EWS patients, in whom caution should be exercised.

Central and Peripheral Nervous System Tumors

Neuroblastoma is the most common extracranial solid tumor in infants, with high metastatic potential. Several cases of ovarian involvement in metastatic neuroblastoma have been reported, and this is the childhood cancer that most frequently spreads to the ovaries, with more than 60% of metastatic neuroblastomas showing circulating malignant cells at the time of diagno-

sis [38–40]. Grèze conducted a study in which healthy ovarian tissue (n = 20) was in vitro-contaminated with human neuroblastoma cell lines and subsequently tested for minimal disseminated disease detection by RT-qPCR for tyrosine hydroxylase (TH), paired-like homeobox 2B (PHOX2B), and doublecortin (DCX) genes. These authors found PHOX2B to be a reliable marker for neuroblastoma cells within ovarian tissue, while TH and DCX were also detected in uncontaminated ovarian tissue, leading to false-positive results, so they cannot be used for this purpose [39]. However, to date, no studies have been published on minimal disseminated disease evaluation of ovarian tissue from patients diagnosed with neuroblastoma.

Conclusion

In conclusion, the pathology that carries the highest risk of reseeding malignant cells upon transplantation is leukemia. Ovarian tissue transplantation is therefore not recommended in these patients. Nevertheless, cryopreservation of ovarian tissue at the time of disease remission, combined with thorough tissue evaluation using sensitive techniques, did allow safe and successful tissue reimplantation in a patient with acute myeloid leukemia. Regarding all other pathologies, even those considered to be low risk, safety cannot be guaranteed, so meticulous tissue examination and informed consent are essential before proceeding with ovarian tissue transplantation.

Practical Clinical Tips

Leukemia is at highest risk of malignant cell contamination, and the absence of malignant cells should be checked carefully in these patients. If all the safety tests prove negative, some fragments can be cautiously grafted back, while in case the tests reveal a risk, individual follicles can be isolated, purged, and subsequently grafted back to patients inside a scaffold (artificial ovary) [9].

Caution is required in case of non-Hodgkin's lymphoma, and histology and anti-CD20 immunohistochemistry are recommended. Similarly, systematic analysis should be encouraged in ovarian tissue of breast cancer patients, using both immunohistochemistry and molecular tools prior to grafting, irrespective of disease stage.

Concerning ovarian cancer and BOT patients, it is not possible to exclude development of new malignant cells from grafted ovarian tissue; therefore, removing and refreezing the grafted tissue could be a new way of managing these patients at risk of recurrence [27].

The overall risk of ovarian metastases in Hodgkin lymphoma and soft tissue and bone sarcomas, with the exception of Ewing sarcoma, may be considered low and autotransplantation safe.

Take-Home Message

Ovarian tissue transplantation allows for restoration in cancer patients, but a multidisciplinary approach, thorough preimplantation evaluation, and informed consent are necessary prior to transplantation. Leukemia carries the highest risk, and transplantation is not recommended, but performing cryopreservation of ovarian tissue at the time of disease remission, combined with thorough tissue evaluation using sensitive techniques, may give a chance of safe reimplantation in patients with acute leukemia. Regarding all other pathologies, even those considered to be at low risk, safety cannot be guaranteed, so meticulous tissue examination is essential before proceeding with ovarian tissue transplantation.

Key Reading

- Transplantation of ovarian tissue allows for restoration of fertility in young cancer patients, but tissue examination and informed consent are essential before proceeding with reimplantation.

References

1. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2017;377(17):1657–65.
2. Dolmans MM, Masciangelo R. Risk of transplanting malignant cells in cryopreserved ovarian tissue. Minerva Ginecol. 2018;70(4):436–43.
3. American Cancer Society. Key statistics for ovarian cancer; 2018 [internet]. www.cancer.org/cancer/ovarian-cancer/about/key-statistics.html.
4. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, Slyusarevsky E, Amariglio N, Schiff E, Rechavi G, Nagler A, Ben Yehuda D. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23:1007–13.
5. Dolmans MM, Marinescu C, Saussoy P, Van Langendonckt A, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood. 2010;116:2908–14.
6. Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sørensen SD, Rosendahl M, Ralfkiaer E, Andersen CY. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. Blood. 2012;120(22):4311–6.
7. Rosendahl M, Andersen MT, Ralfkiær E, Kjeldsen L, Andersen MK, Andersen CY. Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. Fertil Steril. 2010;94:2186–90.
8. Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, Schiff E, Orvieto R, Meirow D. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. Fertil Steril. 2018;109:48–53.
9. Dolmans MM, Amorim CA. Construction and use of artificial ovaries. Reproduction. 2019.REP-18-0536.R2.
10. Soares M, Sahrari K, Amorim CA, Saussoy P, Donnez J, Dolmans MM. Evaluation of a human ovarian follicle isolation technique to obtain disease-free follicle suspensions before safely grafting to cancer patients. Fertil Steril. 2015;104(3):672–80.e2.
11. Soares M, Saussoy P, Maskens M, Reul H, Amorim CA, Donnez J, Dolmans MM. Eliminating malignant cells from cryopreserved ovarian tissue is possible in leukaemia patients. Br J Haematol. 2017;178(2):231–9.
12. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril. 2013;99:1514–22.
13. Kim SS, Radford J, Harris M, Varley J, Rutherford AJ, Lieberman B, Shalet S, Gosden R. Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. Hum Reprod. 2001;16:2056–60.
14. Meirow D, Ben Yehuda D, Prus D, Poliack A, Schenker JG, Rachmilewitz EA, et al. Ovarian tissue banking in patients with Hodgkin's disease: is it safe? Fertil Steril. 1998;69:996–8.
15. Schüring AN, Fehm T, Behringer K, Goeckenjan M, Wimberger P, Henes M, Henes J, Fey MF, Von Wolff M. Practical recommendations for fertility preservation in women by the FertiProTeKT network. Part I: indications for fertility preservation. Arch Gynecol Obstet. 2018;297:241–55.
16. Seshadri T, Gook D, Lade S, Spencer A, Grigg A, Tiedemann K, et al. Lack of evidence of disease contamination in ovarian tissue harvested for cryopreservation from patients with Hodgkin lymphoma and analysis of factors predictive of oocyte yield. Br J Cancer. 2006;94:1007–10.
17. Bittinger SE, Nazaretian SP, Gook DA, Parmar C, Harrup RA, Stern CJ. Detection of Hodgkin lymphoma within ovarian tissue. Fertil Steril 2011;95:803.e3–6.
18. National Cancer Institute - Surveillance, epidemiology, and end results (Seer) Program. epidemiology and end results Program; 2019 [internet]. available from: https://seer.cancer.gov/statfacts/html/breast.html
19. Kyono K, Doshida M, Toya M, Sato Y, Akahira J, Sasano H. Potential indications for ovarian autotransplantation based on the analysis of 5571 autopsy finding of females under the age of 40 in Japan. Fertil Steril. 2010;1:2429–30.
20. Rosendahl M, Timmermans Wielenga V, Nedergaard L, Kristensen SG, Ernst E, Rasmussen PE, Anderson M, Schmidt KT, Andersen CY. Cryopreservation of ovarian tissue for fertility preservation: no evidence of malignant cell contamination in ovarian tissue from patients with breast cancer. Fertil Steril. 2011;95:2158–61.
21. Sánchez-Serrano M, Novella-Maestre E, Roselló-Sastre E, Camarasa N, Teruel J, Pellicer A. Malignant cells are not found in ovarian cortex from breast cancer patients undergoing ovarian cortex cryopreservation. Hum Reprod. 2009;24:2238–43.
22. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Sanchez Serrano M, Schmidt KT, Ernst E, Luyckx V, Andersen CY. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil Steril. 2013;99:1503–13.

23. Luyckx V, Durant JF, Camboni A, Gilliaux S, Amorim CA, Van Langendonckt A, Irenge LM, Gala JL, Donnez J, Dolmans MM. Is transplantation of cryopreserved ovarian tissue from patients with advanced-stage breast cancer safe? a pilot study. J Assist Reprod Genet. 2013;30:1289–99.
24. Bockstaele L, Boulenouar S, van Den Steen G, Dechène J, Tsepelidis S, Craciun L, Noël JC, Demeestere I. Evaluation of quantitative polymerase chain reaction markers for the detection of breast cancer cells in ovarian tissue stored for fertility preservation. Fertil Steril. 2015;104:410–7.
25. American Cancer Society. What are the key statistics for childhood leukemia? 2018 [internet]; 2019. www.cancer.org/cancer/leukemia-in-children/about/key-statistics.html.
26. Dittrich R, Hackl J, Lotz L, Hoffmann I, Beckmann MW. Pregnancies and live births after 20 transplantations of cryopreserved ovarian tissue in a single center. Fertil Steril. 2015;103:462–8.
27. Kristensen SG, Giorgione V, Humaidan P, Alsbjerg B, Bjørn AB, Ernst E, Andersen CY. Fertility preservation and refreezing of transplanted ovarian tissue-a potential new way of managing patients with low risk of malignant cell recurrence. Fertil Steril. 2017;107(5):1206–13.
28. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobling T. First reported clinical pregnancy following hetero- topic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. Hum Reprod. 2013;28:2996–9.
29. Stern CJ, Gook D, Hale LG, Agresta F, Oldham J, Rozen G, Jobliing T. Delivery of twins following heterotopic grafting of frozen-thawed ovarian tissue. Hum Reprod 2014;29:1828.
30. Lotz L, Montag M, Van der Ven H, Von Wolff M, Mueller A, Hoffmann I, Wachter D, Beckmann MW, Dittrich R. Xenotransplantation of cryopreserved ovarian tissue from patients with ovarian tumors into SCID mice - no evidence of malignant cell contamination. Fertil Steril. 2011;95:2612–4.
31. Masciangelo R, Bosisio C, Donnez J, Amorim CA, Dolmans MM. Safety of ovarian tissue transplantation in patients with borderline ovarian tumors. Hum Reprod. 2018;33:212–9.
32. Kim SS, Hwang IT, Lee HC. Heterotopic autotransplantation of cryobanked human ovarian tissue as a strategy to restore ovarian function. Fertil Steril. 2004;82:930–2.
33. Kim SS, Lee WS, Chung MK, Lee HC, Lee HH, Hill D. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. Fertil Steril. 2009;91:2349–54.
34. Kim SS. Assessment of long-term endocrine function after transplantation of frozen-thawed ovarian tissue to the heterotopic site: 10 year longitudinal follow-up study. J Assist Reprod Genet. 2012;29:489–93.
35. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, Liebenthron J, Pellicer A, Donnez J, Andersen CY. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril. 2021;115:1102–15.
36. Dolmans MM, Iwahara Y, Donnez J, Soares M, Jl V, Amorim CA, Poirel H. Evaluation of minimal disseminated disease in cryopreserved ovarian tissue from bone and soft tissue sarcoma patients. Hum Reprod. 2016;31:2292–302.
37. Abir R, Feinmesser M, Yaniv I, Fisch B, Cohen IJ, Ben-Haroush A, Meirow D, Felz C, Avigad S. Occasional involvement of the ovary in Ewing sarcoma. Hum Reprod. 2010;25(7):1708–12.
38. Burchill SA, Lewis IJ, Abrams KR, Riley R, Imeson J, Pearson AD, Pinkerton R, Selby P. Circulating neuroblastoma cells detected by reverse transcriptase polymerase chain reaction for tyrosine hydroxylase mrna are an independent poor prognostic indicator in stage 4 neuroblastoma in children over 1 year. J Clin Oncol. 2001;19:1795–801.
39. Grèze V, Brugnon F, Chambon F, Halle P, Canis M, Amiot C, Grémeau AS, Pereira B, Yáñez Peralta Y, Tchirkov A, Kanold J. Highly sensitive assessment of neuroblastoma minimal residual disease in ovarian tissue using RT-qPCR - A strategy for improving the safety of fertility restoration. Pediatr Blood Cancer 2017;64(5).
40. Yáñez Y, Hervás D, Grau E, Oltra S, Pérez G, Palanca S, et al. TH and DcX mRNAs in peripheral blood and bone marrow predict outcome in metastatic neuroblastoma patients. J Cancer Res Clin Oncol. 2016;142:573–80.