

Cancer cell contamination and decontamination methods for ovaries and testes: special focus on prepubertal gonads with a view to safe fertility restoration

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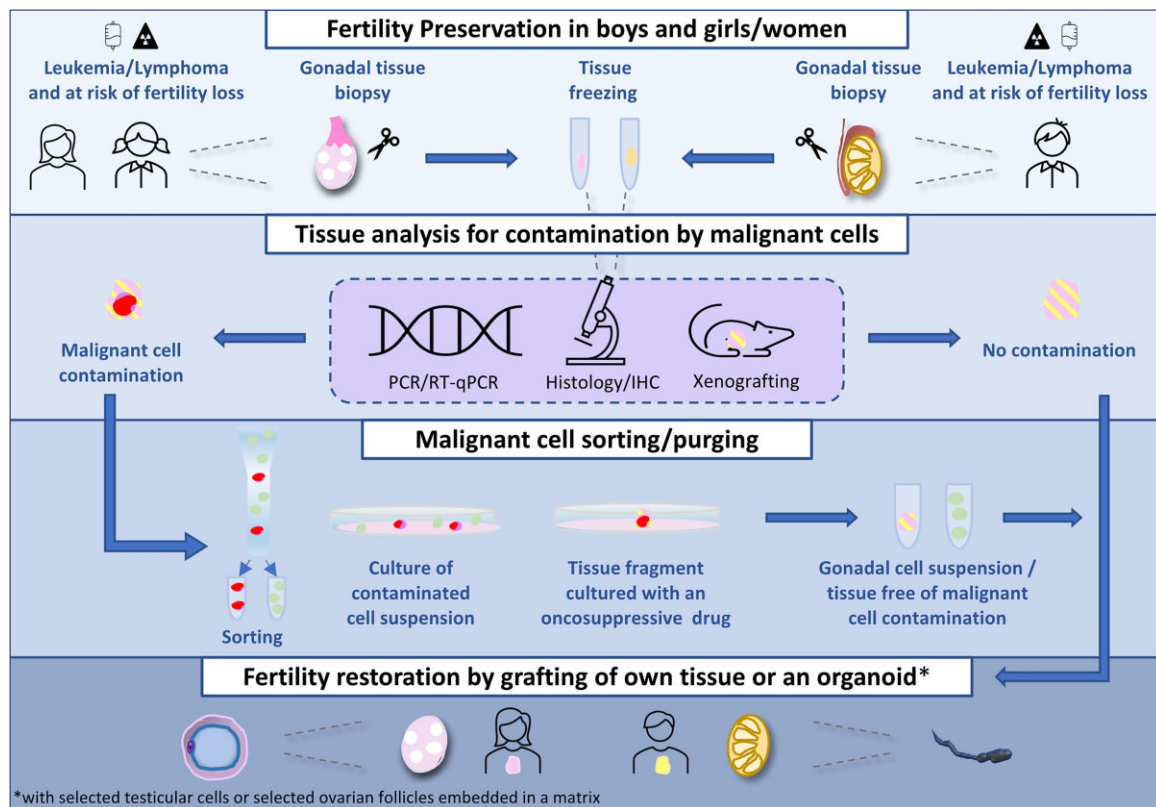
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ABSTRACT: Fertility restoration in patients that survived a hematological cancer during childhood is a core part of their care pathway. Nonetheless, there might be a risk of contamination of the gonads by cancer cells, especially in patients presenting with leukemia and lymphoma. When only a few cancer cells have reached the gonad, they may not be detected by routine histological examination, and therefore more sensitive techniques are required before being confident of the safety of transplanting cryostored testicular and ovarian tissues or cells back to the patient after recovery. Furthermore, if neoplastic cells are identified in the gonadal tissue, methods to eliminate such cells are urgently awaited as the presence of only a few cancer cells may induce disease relapse in these patients. In this review, contamination rates of human gonadal tissue in the case of leukemia or lymphoma as well as decontamination methods applied to both adult and prepubertal testicular and ovarian tissues are presented. Prepubertal gonads will be the main focus as we aim to show how far we have come in establishing safe approaches to fertility restoration. Advances have been made using animal tissue that is usually artificially contaminated by the addition of cancer cell lines to the gonadal cells or tissue, but these techniques need to be improved and still await development in the case of *in vivo* cancer cell invasion of tissue.

Key words: oncofertility / fertility preservation / boys / girls / leukemia / lymphoma / malignant cell contamination / purging / artificial testis / artificial ovary

GRAPHICAL ABSTRACT



Contamination by cancer cells and decontamination methods in testicular and ovarian tissue of patients diagnosed with a hematological malignancy.

Introduction

Leukemia is the most common cancer in the pediatric population especially in the 0–4 years age group where it represents more than 36% of all cancers. Lymphoma on the other hand represents only 5.3% of cases in that age group but increases to 22.5% between 15 and 19 years of age (Steliarova-Foucher et al., 2017). These numbers are on the rise, as an increase of 0.7% in leukemia cases was observed between 2009 and 2018 in the USA and between 1970 and 1999 in Europe (Steliarova-Foucher et al., 2005; Siegel et al., 2020). Fortunately, survivorship is also on the rise with a remission rate estimated at more than 80% in childhood cancers (Gatta et al., 2009).

While these increasing survival rates are mainly the result of effective chemo- and radiotherapeutic treatments, their gonadotoxic effects can lead to sterility, causing additional burden to these patients who have already gone through the traumatic experience of a cancerous disease (Wallace, 2011; Wyns et al., 2015).

For prepubertal boys, unlike adolescent boys and adult men who can benefit from semen cryopreservation, cryostorage of immature testicular tissue (ITT) containing spermatogonial stem cells (SSCs) is the only option to offer these patients a chance of fathering their own genetic child (Wyns et al., 2011, 2020; Kanbar et al., 2021). Potential

indications were proposed back in 2010 and several methods have been investigated to reproduce spermatogenesis with cryopreserved ITT, namely IVM or transplantation of SSCs or ITT fragments (Wyns et al., 2010).

For prepubertal girls, cryopreservation of ovarian cortex followed by its autografting later in life is the only fertility preservation (FP) method so far. Transplantation of cryopreserved ovarian cortex from adult patients is now considered a validated method (Jadoul et al., 2017; Practice Committee of the American Society for Reproductive Medicine. Electronic address: asrm@asrm.org, 2019), with pregnancy rates of around 30%, and it has also already proven successful with prepubertal tissue although the experience remains limited in the pediatric population (Demeestere et al., 2015; Matthews et al., 2018; Rodriguez-Wallberg et al., 2021). Other methods to restore fertility with cryostored ovarian tissue, whether post- or prepubertal, for example *in vitro* growth and maturation of follicles contained in the tissue (Anderson et al., 2014), are still at the research stage (Fig. 1).

Despite progress in developing new methods of FP, a major concern is the possibility of malignant cell contamination of cryostored ovarian and testicular tissues, especially in the case of hematological malignancies, with the risk of causing disease relapse if auto-transplanted (Hou et al., 2007a; Meirow et al., 2008; Shapira et al., 2018).

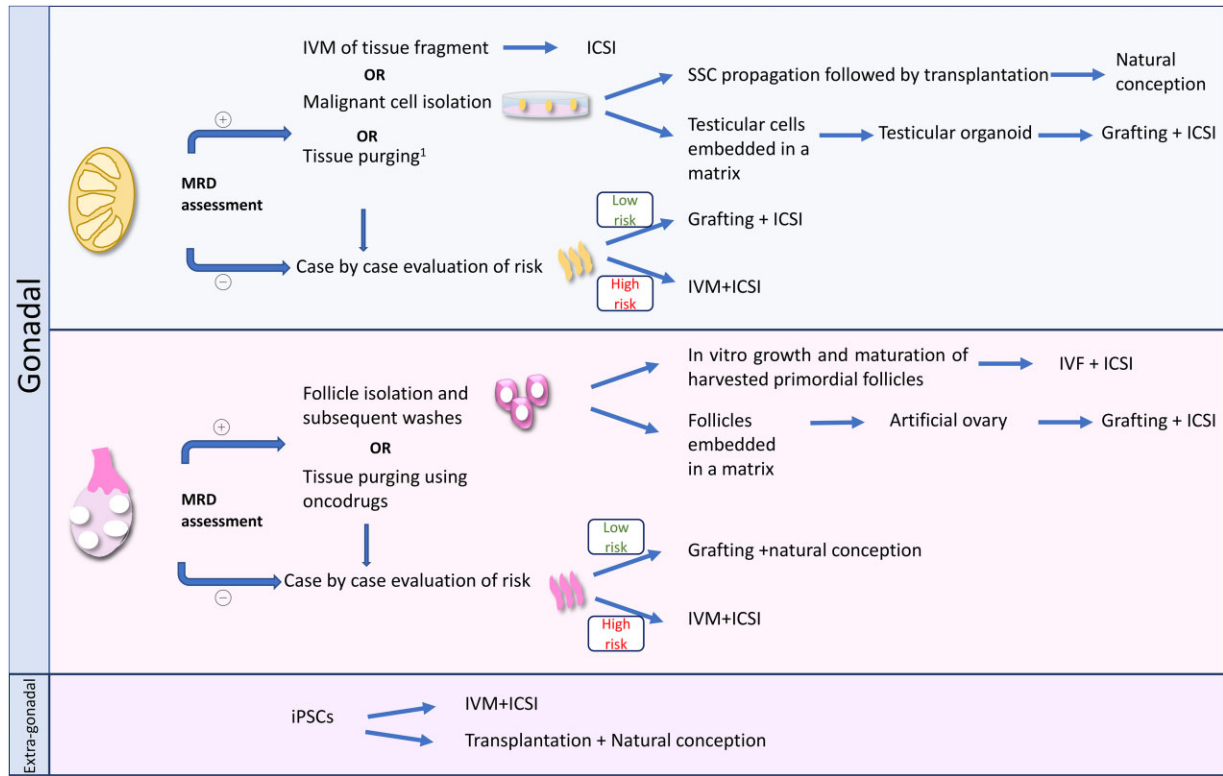


Figure 1. Options for safe fertility restoration in cancer survivors. For prepubertal patients, gonadal tissue is cryopreserved either before or after the initiation of gonadotoxic treatment. After assessing the tissue for presence of minimal residual disease (MRD), in the case of absence of MRD it can be transplanted back to the patient to allow an *in vivo* maturation and restoration of fertility. If cancer cells are encountered during tissue evaluation, or if the tissue is considered to be at high risk for presence of malignant cells, the germ cells can be isolated and matured *in vitro*, with or without embedding in a matrix. A third option is the use of extra-gonadal cells (iPSCs) to safely generate germ cells. SSC, spermatogonial stem cells; iPSCs, induced pluripotent stem cells; IHC, immunohistochemistry. ¹Technique not previously applied to the testis (upper panel).

Reasons for gonadal tropism of cancer cells are poorly understood and differ between the ovary and testis. In this regard, males with acute lymphoblastic leukemia (ALL) have a higher rate of relapse in the testicle than girls in the ovary (Miller and Miller, 1983).

Several hypotheses have been proposed to explain such propensities. On the one hand, estrogens seem to have a leukemogenesis effect (Gralnick and Dittmar, 1969; Schnyder et al., 2009), favoring the proliferation of metastasizing cells in the ovary. On the other hand, disease patterns specific to male hematological cancers (i.e. a higher rate of T-cell pathologies causing higher leucocyte counts, organ, and nodal involvement) (Miller, 1980; Sather et al., 1981; Inaba and Pui, 2021) may play a role, together with other factors. Among those are the high proliferative activity of testicular cells, molecules released by Leydig cells that were shown to stimulate lymphocyte proliferation *in vitro* (e.g. substance P and methionine enkephalin), and the well-known marked immunosuppression within the testis that could be responsible for an ineffective immunological response against leukemic cells (Pollanen et al., 1990; Ritzén, 1990).

Improving knowledge on gonadal contamination rates to obtain best estimates of risks and developing strategies to rid gonads of

contaminating cancer cells are the major challenges of FP and restoration methods.

The aim of this review is to present knowledge on gonadal contamination rates following hematological malignancies in prepubertal patients as well as decontamination strategies for gonadal tissues aimed at establishing safe approaches to fertility restoration. To provide the clinician with an exhaustive overview of the subject, including perspectives for prepubertal tissue application, data on adult gonads have been included, especially with regards to novel decontamination technologies, which are mainly studied using adult gonadal tissue.

Methods

The search was conducted for all articles published until April 2022 in the MEDLINE database. The following search queries relating to the subject were run on the PubMed search engine: acute lymphoblastic leukemia OR lymphoma AND testicular cells; contamination AND testicular cells; malignant cells AND gonadal contamination; ovary AND (cancer OR malignant) cells AND (leukemia OR lymphoma).

Original papers and review articles in English and French reporting on contamination rates and decontamination methods for both the testis and ovary in prepubertal patients suffering from leukemia and lymphomas were included. With regards to contamination rates, case reports were excluded. When the pubertal status was not specified, data on girls older than 12 years were considered post-pubertal. Studies on adult tissue were included for their relevance in clinical practice and when data on prepubertal gonads was not available.

Figure 2 presents the flowchart describing the articles selection process and outcome.

What are the reported cancer cell contamination rates of gonads by disease?

The overall contamination rates (by both leukemia and lymphoma cancer cells) in the testis and ovary of prepubertal patients are, respectively, 37% ($n = 164/448$) and 12% ($n = 63/531$) (Tables I and II). Similar rates are reported in adult ovaries in Table III. Hereafter, the rates are presented in detail based on the nature of the hematological

malignancy, with a specific focus on studies reporting results from tissues taken for FP purposes.

Testis

Leukemia

Multiple studies were conducted in the 1980s and 1990s to determine contamination rates in the testis following chemotherapy for ALL. Their main aim was to demonstrate that the testis was a preferential organ for leukemic relapse. It was hypothesized that owing to its specific architecture, with a lack of fenestration between endothelial cells, the testis was less sensitive to chemotherapy and thus a sanctuary for leukemic cells (Ritzén, 1990). Overall, in prepubertal boys, contamination rates ranged from 21% to 100% (Givler, 1969; Kamiyama and Funata, 1976; Reid and Marsden, 1980; Wong et al., 1980; Kim et al., 1981; Thomas et al., 1982; Ortega et al., 1984; Chessells et al., 1986; Layfield et al., 1988; Akhtar et al., 1991; Wilkins et al., 1992; de Almeida et al., 1994). Table I summarizes the studies in prepubertal boys, including the timing and methods of cancer cell detection, and the type of tissue sampling.

The highest infiltration rates were observed in postmortem studies (Givler, 1969; Kamiyama and Funata, 1976; Reid and Marsden, 1980)

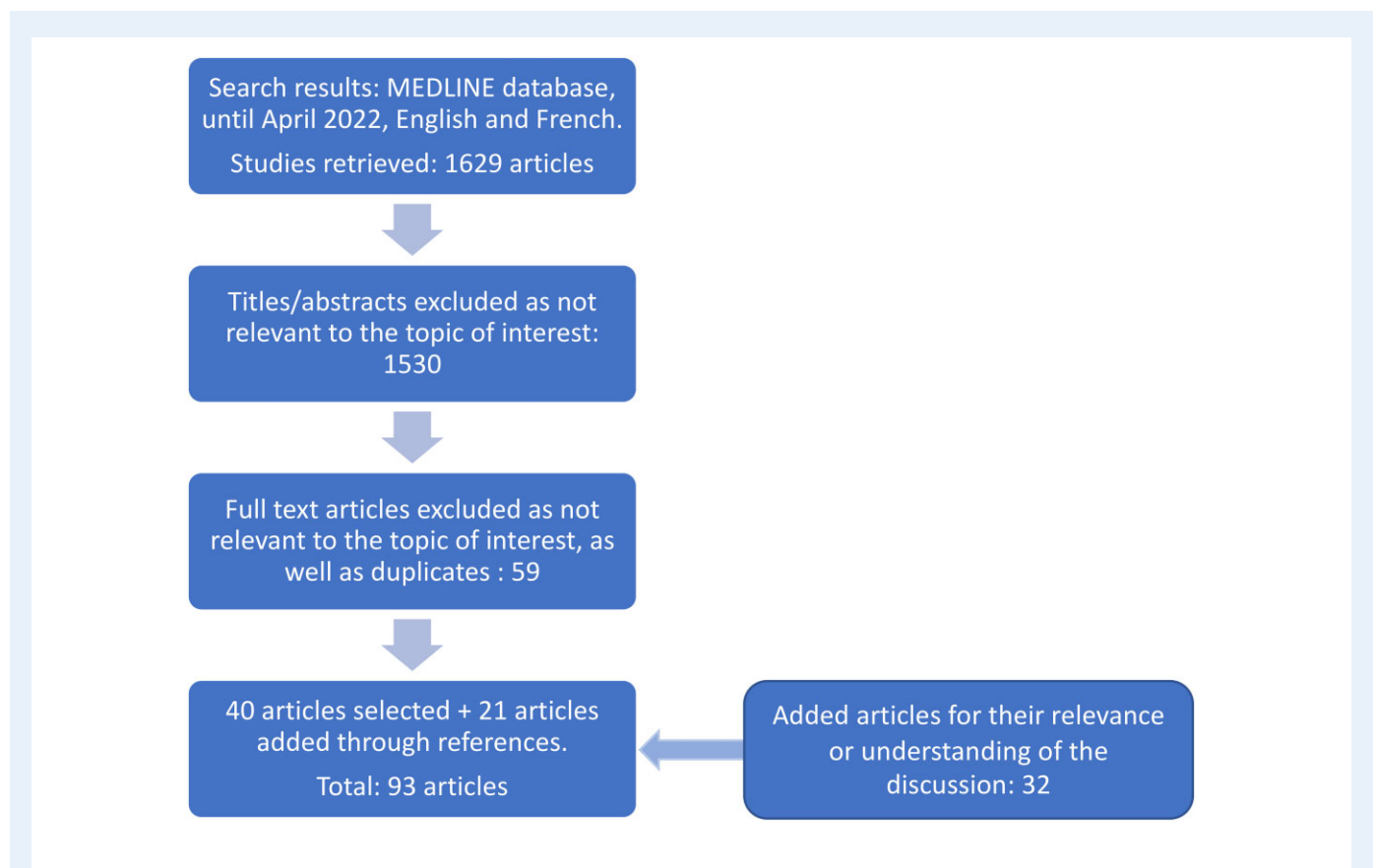


Figure 2. General flowchart of article selection process. The numbers of articles found per query were as follows: for acute lymphoblastic leukemia OR lymphoma AND testicular cells: 620 articles; for contamination AND testicular cells: 289 articles; for malignant cells AND gonadal contamination: 68 articles; and for ovary AND (cancer OR malignant) cells AND (leukemia OR lymphoma) (filter applied: Human): 652 articles. Thirteen articles, 15 articles, and 10 articles were reported on prepubertal, adult, and on both prepubertal and adult tissues, respectively.

Table 1 Cancer cell contamination rates in prepubertal testes.

Reference	Number of patients	Time of detection	Sampling method	Number of patients with cancer cells at histology	Number of patients with cancer cells at IHC	Number of patients with a clinical suspicion of infiltration of infiltration (testis enlargement)	Overall contamination rate (% of patients) ¹
Leukemia							
(Givler, 1969)	8	Postmortem	Unilateral wedge biopsy	8/8	NA	8/8	100%
(Kamiyama and Funata, 1976)	7	Postmortem	Whole testis	5/7	NA	Not specified	71%
(Reid and Marsden, 1980)	50	Postmortem	Whole testis	38/50	NA	Not specified	76%
(Wong et al., 1980)	29	In remission (>30 months)	Bilateral wedge biopsy	12/29	NA	5/29	41%
(Kim et al., 1981)	24	At initial diagnosis	Bilateral wedge biopsy	5/24	NA	0/24	21%
(Thomas et al., 1982)	25	During maintenance therapy	Wedge biopsy ²	10/25	16/25	0/25	68%
(Ortega et al., 1984)	68	In remission (at an average of 29 months)	Bilateral wedge biopsy	23/68	NA	7/68	34%
(Chessells et al., 1986)	37	At the end of treatment	Unilateral wedge biopsy	6/37	6/37	4/37	21%
(Layfield et al., 1988)	11	At the end of treatment	Bilateral wedge biopsy and bilateral FNAB	5/11	NA	0/11	45%
(Akhtar et al., 1991)	70	Not specified	Bilateral FNAB	NA*	NA	NA	NA*
(Wilkins et al., 1992)	28	Post-treatment after 2 years (n = 22) or in case of testicular enlargement (n = 7)	Wedge biopsy ²	12/28	11/12	7/28	43%
(de Almeida et al., 1994)	31	At the end of treatment (n = 20) or in case of suspicion of testicular relapse (n = 11)	Bilateral FNAB	11/31	NA	13/31	35%
Non-Hodgkin's lymphoma + leukemia							
(Hayder et al., 1988)	20	In remission ³ (n = 18) or in case of testicular enlargement (n = 2)	Bilateral FNAB	3/20	NA	2/20	15%
(Suc et al., 1989)	30	At the end of treatment (8–48 months after diagnosis) (n = 25), or in cases of testicular enlargement (n = 4) or meningeal relapse (n = 1)	Bilateral wedge biopsy	5/30	12/30	4/30	40%
Hodgkin's lymphoma							
(Reid and Marsden, 1980)	10	Postmortem	Whole testis	5/10	NA	Not specified	50%
Total	448	NA	NA		164¹		37%¹

FNAB, fine-needle aspiration biopsy; NA, not applicable.

*34/106 (samples) of 70 boys were positive at histology (32%); number of patients with positive samples unknown.

¹Calculated based on the positivity per patient for at least one technique.

²Laterality not specified.

³Duration of follow-up unknown.

Table II Cancer cell contamination rates in prepubertal ovaries (girls <12 years of age).

Reference	Number of patients	Time of detection and sampling method	Number of patients with cancer cells at histology	Number of patients with cancer cells at IHC	Number of patients with cancer cells at multi-color flow cytometry	Number of patients with cancer cells detected with molecular biology	Tumor development after tissue xenotransplantation	Overall contamination rate (%) ¹
Leukemia								
(Kamiyama and Funata, 1976)	7	Whole ovary, postmortem	2/7	NA	NA	NA	NA	28%
(Reid and Marsden, 1980)	27	Whole ovary, postmortem	15/27	NA	NA	NA	NA	55%
(Poirot et al., 2007)	6	On fresh cortical ovarian fragments, after induction chemotherapy	0/6	NA	NA	NA	NA	0%
(Dolmans et al., 2010)	3	On frozen thawed cortical ovarian fragments banked after one round of methotrexate and cortisone	0/3	NA	NA	1/2	1/3	33%
(Rosendahl et al., 2010)	3	On frozen thawed cortical ovarian fragments, banked prior to treatment	0/3	0/3	NA	2/3	NA	66%
(Kyono et al., 2010)	392	Autopsy*	Not specified	Not specified	Not specified	Not specified	Not specified	7.9%
(Greve et al., 2012)	8	On frozen thawed cortical ovarian fragments, complete remission	0/7	0/7	NA	2/2	0/8	25%
(Asadi-Azarbaijani et al., 2016)	6	On frozen thawed cortical fragments, banked after chemotherapy	NA	NA	NA	2/6	NA	33%
(Zver et al., 2022)	3	On frozen thawed cortical fragments, banked after chemotherapy	NA	NA	0/3	NA	0/3	0%
Lymphoma								
(Kyono et al., 2010)	76	Autopsy*	Not specified	Not specified		Not specified	Not specified	10.5%
Total	531	NA			63¹			12%¹

NA, not applicable; IHC, immunohistochemistry.

*Sampling method is not specified.

¹ Calculated based on the positivity per patient for at least one technique.

Table III Cancer cell contamination rates in adult ovaries.

Reference	Number of patients	Time of detection and sampling method	Number of patients with cancer cells at histology	Number of patients with cancer cells at IHC	Number of patients with cancer cells at multi-color flow cytometry	Number of patients with cancer cells detected with molecular biology	Tumor development after tissue transplantation	Overall contamination rate (%) ¹
Leukemia								
(Kamiyama and Funata, 1976)	24	Whole ovary, postmortem	16/24	NA	NA	NA	NA	67%
(Poirot, Martelli <i>et al.</i> , 2007)	6	On fresh cortical ovarian fragments, after induction chemotherapy	0/6	NA	NA	NA	NA	0%
(Meirow, Hardan <i>et al.</i> , 2008)	9	On frozen thawed cortical ovarian fragments, after chemotherapy (n = 8), prior to treatment (n = 1)	0/9	NA	NA	1/2	NA	11%
(Dolmans, Marinescu <i>et al.</i> , 2010)	15	On frozen thawed cortical ovarian fragments, banked after one round of methotrexate and cortisone	0/15	NA	NA	8/14	4/15	60%
(Rosendahl, Andersen <i>et al.</i> , 2010)	23	On frozen thawed cortical ovarian fragments, banked prior to treatment	0/23	0/23	NA	4/5	NA	17%
(Kyono, Doshida <i>et al.</i> , 2010)	1635	Autopsy*	Not specified	Not specified	Not specified	Not specified	Not specified	8.6%
(Greve, Clasen-Linde <i>et al.</i> , 2012)	17	On frozen thawed cortical ovarian fragments, after complete remission	0/17	0/17	NA	2/5	0/24	12%
(Asadi-Azarbaijani, Sheikhi <i>et al.</i> , 2016)	8	On frozen thawed cortical ovarian fragments, banked before chemotherapy (n = 4), after chemotherapy (n = 4)	NA	NA	NA	4/8	NA	50%
(Meirow, Ra'anani <i>et al.</i> , 2016)	2	On frozen thawed cortical ovarian fragments, banked after chemotherapy (n = 1), before chemotherapy (n = 1)	0/2	0/1	NA	0/2	0/1	0%
(Silber, DeRosa <i>et al.</i> , 2018)	3	On frozen thawed cortical fragments, after remission	0/3	0/3	NA	NA	NA	0%
(Chevillon, Clappier <i>et al.</i> , 2021)	30	On frozen thawed cortical and medullary ovarian fragments, after complete remission	NA	NA	NA	9/30	NA	30%
(Zver, Frontczak <i>et al.</i> , 2022)	12	On frozen thawed cortical ovarian fragments, after at least one round of chemotherapy (n = 11), before chemotherapy (n = 1)	NA	NA	5/12	0/3	0/12	42%
Subtotal leukemia	1784	NA			190 ¹			10.7% ¹
Hodgkin's lymphoma								
(Kim, Radford <i>et al.</i> , 2001)	13	On frozen thawed cortical tissue, harvested prior to chemotherapy	0/13	NA	NA	NA	0/13	0%

(continued)

Table III Continued

Reference	Number of patients	Time of detection and sampling method	Number of patients with cancer cells at histology	Number of patients with cancer cells at IHC	Number of patients with cancer cells at multi-color flow cytometry	Number of patients with cancer cells detected with molecular biology	Tumor development after tissue transplantation	Overall contamination rate (%) ¹
(Seshadri, Gook <i>et al.</i> , 2006)	26	On frozen thawed ovarian cortex and medulla, prior to chemotherapy (n = 9), after chemotherapy (n = 17)	0/26	0/26	NA	NA	NA	0%
(Meirow, Hardan <i>et al.</i> , 2008)	33	On frozen thawed ovarian cortex, banked before chemotherapy (n = 17), after chemotherapy (n = 16)	0/33	0/1	NA	NA	0/1	0%
(Hoekman, Smit <i>et al.</i> , 2015)	6	On frozen thawed ovarian cortex banked prior to chemotherapy (n = 5), after chemotherapy (n = 1)	0/6	0/6	NA	NA	NA	0%
(Meirow, Ra'anani <i>et al.</i> , 2016)	9	On frozen thawed ovarian cortex, banked prior to chemotherapy (n = 3), after chemotherapy (n = 6)	0/9	0/9	NA	NA	NA	0%
(Silber, DeRosa <i>et al.</i> , 2018)	4	On frozen thawed ovarian cortex, timing of banking not specified	0/4	0/4	NA	NA	NA	0%
Non-Hodgkin's lymphoma								
(Kim, Radford <i>et al.</i> , 2001)	5	On frozen thawed ovarian cortex banked prior to chemotherapy	0/5	NA	NA	NA	0/5	0%
(Meirow, Hardan <i>et al.</i> , 2008)	14	On frozen thawed ovarian cortex, banked prior to chemotherapy (n = 5), after chemotherapy (n = 9)	0/14	NA	NA	0/2	0/1	0%
(Hoekman, Smit <i>et al.</i> , 2015)	2	On frozen thawed ovarian cortex banked prior to chemotherapy	0/2	0/2	NA	NA	NA	0%
(Meirow, Ra'anani <i>et al.</i> , 2016)	4	On frozen thawed ovarian cortex, banked prior to chemotherapy (n = 2), after chemotherapy (n = 2)	0/4	0/4	NA	NA	NA	0%
Non-Hodgkin's lymphoma + Hodgkin's lymphoma								
(Kyono, Doshida <i>et al.</i> , 2010)	660 ²	Autopsy*	Not specified	Not specified	NA	Not specified	Not specified	13.6%
Subtotal lymphoma	776	NA			90 ¹			11.6% ¹
Total	2560	NA			280¹			11%¹

*Sampling method is not specified.

NA, not applicable; IHC, immunohistochemistry.

¹Calculated based on the positivity per patient for at least one technique.²Type of lymphoma within the cohort not specified.

or when there was a suspicion of clinical infiltration (Akhtar *et al.*, 1991; Wilkins *et al.*, 1992; de Almeida *et al.*, 1994), i.e. testicular enlargement. Hence, these studies are indicative of the proliferation potential of leukemic cells and of infiltration rates at late stages of the disease. Contamination rates were also assessed at different stages of the care pathway, i.e. at remission or during maintenance therapy; lower rates (respectively, 41% and 34%) were observed during remission phases and, thus, after therapy (Wong *et al.*, 1980; Ortega *et al.*, 1984) in comparison to patients that were receiving maintenance chemotherapy, where invasion rates were as high as 68% (Thomas *et al.*, 1982).

Only one study (Kim *et al.*, 1981) assessed testicular contamination at initial diagnosis, including patients with ALL, prior to any treatment. All testes were of normal size without clinical suspicion of leukemic infiltration. The authors used the presence of leukemic cells as an indicator of the 'body burden of the tumor' and thus as a prognostic factor. Based on histology showing rare focal clusters of lymphoblasts between seminiferous tubules, 21% ($n = 5/24$) of the patients presented with testicular leukemic infiltration.

Sampling methods are key when assessing cancer cell contamination rates. For wedge biopsies, invasion rates ranged from 19% to 68% with an average of 39% (Wong *et al.*, 1980; Kim *et al.*, 1981; Thomas *et al.*, 1982; Ortega *et al.*, 1984; Chessells *et al.*, 1986; Wilkins *et al.*, 1992), while when using fine-needle aspiration biopsy (FNAB), a technique that encompasses smaller amounts of tissue, 32% of specimens appeared to be infiltrated by leukemic cells (Akhtar *et al.*, 1991).

Different figures were also found when comparing detection techniques, for example classical histology versus immunohistochemistry (IHC: known to be more sensitive than histology in detecting malignant cells) using specific protein markers for cancer cells (Thomas *et al.*, 1982; Chessells *et al.*, 1986; Wilkins *et al.*, 1992). Indeed, using IHC for deoxynucleotidyl transferase (TdT) enzyme (usually detected in more than 97% of childhood ALL cases; Bollum, 1979), Chessells *et al.* (1986), found an overall contamination rate of 21% and concordance between histology and IHC in 35 out of 37 patients. Nonetheless, among these 35 patients, eight whose initial findings with histology and IHC were negative for testicular infiltration relapsed and two of those relapsed in the testis, which points to the risk for false negatives with these techniques. By contrast, Thomas *et al.* (1982) conducted a similar study on a group of 25 patients with ALL whose histological evaluation showed no blast cell infiltration whereas they were all positive for TdT at immunological evaluation. Such discrepancies call for techniques with higher sensitivity to be used, allowing the detection of minimal residual disease (MRD), considered to have significant clinical value at 0.01% or 1 MRD cell in 10 000 cells in bone marrow samples. The application of molecular biology techniques, namely PCR and RT-PCR to search for specific molecular markers of immunoglobulin/T-cell rearrangements and gene rearrangements, respectively, as applied to bone marrow samples during treatment and remission (van Dongen *et al.*, 2003) were not reported for testicular tissue.

Lymphoma

Testicular involvement following lymphoma is a rare occurrence and is usually described in older patients. The studies include mainly adult patients and it is often impossible to determine the numbers of prepubertal boys included in the cohorts as well as some more disease-

specific data (Hodgkin's lymphoma (HL) versus non-Hodgkins's lymphoma (NHL)).

Based on histology at autopsy, Givler (1969) found an infiltration rate of the testis of 18.6% in a series of 102 patients diagnosed with NHL. The exact number of children among the total cohort of this study is, unfortunately, unknown but among the patients with a testicular infiltration five were children. Moreover, none of the 44 patients diagnosed with HL had a cancerous testicular infiltration and it is unclear whether children were included in this cohort.

Only one study (Reid and Marsden, 1980) determined testicular contamination rates in prepubertal patients with HL, where 50% of boys presented with a cancerous infiltration based only on histological evaluation. Of note, this rate is based on postmortem evaluations and on a group of only 10 patients. Studies on testicular contamination rates in prepubertal boys with NHL (Hayder *et al.*, 1988; Suc *et al.*, 1989) were not informative as cohorts comprised patients diagnosed both with ALL and NHL, and only overall rates were presented (15% (Hayder *et al.*, 1988) and 40% (Suc *et al.*, 1989)).

Ovary

Leukemia

Contamination rates in the ovarian tissue of prepubertal patients diagnosed with leukemia varied between 0% and 66% (versus 0–67% in adults, Table III).

Table II summarizes the studies on prepubertal ovaries, including type of sampling, cancer cell detection methods and time of detection.

Necropsy tissue samples were mostly analyzed and showed an overall contamination rate (Kamiyama and Funata, 1976; Reid and Marsden, 1980; Kyono *et al.*, 2010) of 11.3% ($n = 48/426$) compared to 24% ($n = 7/29$) for ovarian tissue collected for FP purposes (Poirot *et al.*, 2007; Dolmans *et al.*, 2010; Rosendahl *et al.*, 2011; Greve *et al.*, 2012; Asadi-Azarbaijani *et al.*, 2016; Zver *et al.*, 2022).

Regarding detection methods, more sensitive techniques, such as PCR and xenotransplantation, have been used in the more recent studies to assess the presence of few leukemic cells (Dolmans *et al.*, 2010; Rosendahl *et al.*, 2010; Greve *et al.*, 2012; Asadi-Azarbaijani *et al.*, 2016; Zver *et al.*, 2022). In the study of Rosendahl *et al.* (2010), histology and IHC did not allow detection of leukemic cells, while in three patients of the cohort in whom genetic markers were identified (two patients with the BCR-ABL (a specific chronic myeloid leukemia translocation) and one with TEL-AML1 (ALL fusion transcript)), PCR showed the presence of residual disease in two of them.

Discrepant results between detection methods (histology, PCR, and xenotransplantation) were also reported in studies assessing whether the reimplantation of cryopreserved ovarian tissue was safe (Dolmans *et al.*, 2010; Greve *et al.*, 2012; Zver *et al.*, 2022).

Greve *et al.* (2012) analyzed samples of eight prepubertal leukemic patients (seven in complete remission and one in the active phase of the disease). No leukemic infiltration was observed at histology while in samples of two patients, both in complete remission, RT-PCR showed some leukemic cells but at the limit of the detection threshold. As xenotransplanted mice did not show any signs of cancer cell invasion, the authors hypothesized that either there were no cancer cells in the grafted tissue or that residual leukemic cells in grafts were non-viable or in too small numbers to cause tumor development. Similarly, using multi-color flow cytometry based on eight leukemia

markers per patient, no malignant cells were identified after multiple rounds of chemotherapy and no tumors developed after xenografting to immunodeficient mice (Zver et al., 2022). By contrast, after only one round of chemotherapy, cryopreserved ovarian tissue from one leukemia patient with positive PCR did induce the malignant disease in mice when xenotransplanted (Dolmans et al., 2010). Despite discrepant results on tissue xenografting, two teams have autotransplanted ovarian tissue from patients with leukemia and no disease relapse was reported to date, whether the tissue was harvested prior or after chemotherapy (Meirow et al., 2016; Silber et al., 2018).

The medulla seems to be most often invaded by leukemic cells compared to the cortex. Indeed, Reid and Marsden (1980) showed that a leukemic invasion was mainly observed in the medulla in patients with ALL, and that four out of nine patients suffering from either acute myeloid leukemia (AML) or acute myelomonocytic leukemia (AMML) also had a medullary mass.

Lymphomas

To our knowledge, only one study assessed the ovarian contamination rate in prepubertal patients diagnosed with lymphoma (Kyono et al., 2010) where 10.5% of girls presented with an infiltration based on autopsy findings. Nonetheless, the study was not informative regarding the patient's type of lymphoma (HL or NHL).

However, in adult and adolescent patients with lymphoma that underwent a FP procedure, several teams investigated thawed ovarian tissue for cancer cell contamination (Kim et al., 2001; Radford et al., 2001; Donnez et al., 2004; Meirow et al., 2005, 2008; Oktay and Oktem, 2010). Some teams harvested the ovarian tissue after chemotherapy (Radford et al., 2001; Meirow et al., 2005, 2008) and showed no infiltration by cancer cells. Meirow et al. (2008) reported the largest cohort of 47 patients diagnosed with either HL or NHL and did not observe cancer cell contamination of the tissue either based on histology or on more sensitive techniques (IHC or PCR) performed for only three of the patients. Other teams harvested the tissue before chemotherapy (Kim et al., 2001; Donnez et al., 2004; Oktay and Oktem, 2010) and found no cancer cells at histological examination. Two teams (Donnez et al., 2004; Oktay and Oktem, 2010) autotransplanted the ovarian tissue back to the patients, who did not relapse. While based on these studies, not receiving chemotherapy prior to tissue harvest did not seem to be a risk factor for ovarian cancer cell contamination and recurrence, the presence of cancer cells was reported using histology and IHC prior to the start of therapy in a 19-year-old woman diagnosed with HL (Bittinger et al., 2011).

What are the available decontamination protocols for gonadal tissue?

Different methods to obtain auto-transplants that are free of cancer cells have been investigated. Because of the different tissue architectures of the male and female gonads, decontamination techniques differ between testis and ovaries.

While culture systems and/or cell sorting techniques using fluorescence activated cell sorting (FACS) or magnetic-activated cell sorting (MACS) to obtain cancer cell-free suspensions have been applied for

testicular tissue, follicle isolation through micropipette pickup followed by washing in droplets of medium or cancer cell purging protocols based on onco-suppressive agents in culture have been investigated for ovaries.

Selected gonadal cells can then either be used after simple embedding in extracellular matrices or after prior development of organoids in culture before transplantation. Further details on the subject are limited to a summary figure on fertility restoration options (Fig. 1) as this is not in the scope of this review.

Decontamination methods applied to testicular tissue

Several studies using non-human testicular tissue attempted decontamination of cell suspensions (Fujita et al., 2005; Geens et al., 2007; Hou et al., 2007b, 2009; Hermann et al., 2011) mainly through FACS. Although some studies showed encouraging results, methods still need to be further perfected as either persistence of cancer cells after sorting or a relapse after xenotransplantation of the sorted cell suspensions were observed (Table IV).

For human tissue, disappointing results were found when using cell sorting techniques (Fujita et al., 2006; Geens et al., 2011; Dovey et al., 2013). On the one hand, Geens et al. (2011) using MACS and matrix adhesion were unable to decontaminate the cell suspensions, as proved by residual cancer cells at FACS and PCR for MRD. On the other hand, Dovey et al. used FACS with specific cell markers to decontaminate testicular cell suspensions that were artificially contaminated by a leukemic cell line (MOLT-4). The sorted cells were xenotransplanted to nude mice and there was no tumor formation (Dovey et al., 2013). Even though at first glance the decontamination protocol seemed effective, the low invasion rate observed in recipient mice of the unsorted fraction (50%) and the low tumor development rate (43%) when pure MOLT-4 cells were xenotransplanted, raise the question of the invasion potential of this cell line.

Cell culture was also attempted to obtain cell suspensions free of cancer cells. Using a culture system designed for the proliferation of SSCs (Sadri-Ardekani et al., 2011), Sadri-Ardekani et al. (2014) demonstrated the viability of SSCs while ridding the cell suspensions of cancer cells. These results were confirmed by the inability of PCR to detect viable ALL cells after 20–26 days of culture at the highest concentration of ALL cells (40%) in the cell suspension. It is of note that all studies attempted decontamination of artificially contaminated cell suspensions where interactions between cancer cells and testicular cells may differ from those occurring during tissue invasion by cancer cells *in vivo*.

Decontamination methods applied to ovarian tissue

Two different approaches, namely isolation of follicles prior to embedding and transplantation, and *in vitro* purging protocols, to obtain cancer-cell-free reproductive material have been studied (Table V).

Peek's group (Mulder et al., 2019; Eijkenboom et al., 2021) proposed a purging protocol for ovarian tissue fragments through the

Table IV Cancer cell decontamination protocols for testes.

Study	Cell selection/purging method	Testicular cells source (n = number of donors)	Malignant cells source	Number of cell suspensions analyzed	Outcome
(Fujita <i>et al.</i> , 2005)	FACS with CD 45 and MHC class I malignant cell markers <u>Protocol:</u> Selection of MHC Class-I +/- CD45 + cells	5-week-old C57BL/6 mice	Murine C1498 leukemia cell line	12 cell suspensions ¹	Allotransplantation after sorting: no tumor development in 12/12 mice. Histology of transplanted testes: no leukemic cells
(Fujita <i>et al.</i> , 2006)	FACS with CD45, HLA-ABC malignant cell markers <u>Protocol:</u> Selection of HLA-ABC+/CD45+ cells	Human testicular tissue from a patient operated on for blunt trauma on the testis (n = 1)	8 human leukemia and lymphoma cell lines 1) Jurkat T-cell leukemia 2) MOLT-4 T-cell leukemia 3) HL60 Promyelocytic leukemia 4) KU812 CML 5) K562 CML 6) U-937 Histiocyte lymphoma 7) RAJI Burkitt lymphoma 8) Diffuse large B-cell non-Hodgkin's lymphoma	8 cell suspensions ¹	Flow cytometry: cancer cells in 1/8 cell lines after sorting Presence of 1.45% of K562 CML cells in the germ cell fraction
(Hou <i>et al.</i> , 2007b)	FACS with CD4, MHC class I for malignant cell markers and EpCAM for germ cells <u>Protocol I:</u> Selection of Ep-CAM positive cells <u>Protocol II:</u> Selection of Ep-CAM + secondary antibody positive cells <u>Protocol III:</u> Depletion of CD4+ and MHC Class I+ cells <u>Protocol IV:</u> Selection of Ep-CAM and depletion of CD4 and MHC Class-I positive cells	PVG rats (n = 40)	Roser's T-cell leukemia	NA	Allotransplantation after sorting: tumor development following protocols I, II, III
(Geens <i>et al.</i> , 2007)	MACS for enrichment of germ cells followed by FACS for decontamination with MHC Class-I malignant cell marker and CD49-f germ cell marker <u>Protocol:</u> Selection of CD-49 f+/MHC-Class-I—cells.	B6CBAFI/Juco mice (n=NA)	EL-4 cells mouse	8 cell suspensions with 5% EL-4 cells	Flow cytometry: EL-4 cells in 6/8 cell suspensions after sorting Cell culture after sorting: tumor cells growth in 3.1% of wells Allotransplantation after sorting: tumor development in 1/20 transplanted mice
	FACS with MHC-Class I malignant cell marker <u>Protocol:</u> Selection of MHC Class I positive cells	Human (n= NA)	SB cells	5 cell suspensions with 5% SB cells 6 cell suspensions with 0.05% SB cells	Flow cytometry: SB cells in 5/5 cell suspensions after sorting Cell culture after sorting: tumor cells growth in 3/5 cultures PCR after sorting: 4/5 positive suspensions (PCR was not performed on one cell suspension) Flow cytometry: SB cells in 6/6 cell suspensions after sorting Cell culture after sorting: tumor cells growth in 5/6 cultures PCR after sorting: 5/6 positive suspensions

(continued)

Table IV Continued

Study	Cell selection/purging method	Testicular cells source (n = number of donors)	Malignant cells source	Number of cell suspen- sions analyzed	Outcome
(Hou et al., 2009)	MACS with CD4 malignant cell marker <u>Protocol</u> : Selection of CD4 positive cells	PVG rats (n = 4)	Roser's T-cell leukemia	4 testicular cell suspensions ¹	Flow cytometry: mean reduction of 29.5% leukemic cells in 4 cell suspensions after sorting Immunocytochemistry after sorting: mean reduction of 12.8% of leukemic cells in 4 cell suspensions Allotransplantation after sorting: tumor development in 20/20 rats
(Hermann et al., 2011)	FACS with CD45 malignant cell marker and CD90 germ cell marker <u>Protocol</u> : Selection of CD45+/CD90—cells	Rhesus monkeys (n = 5)	Human MOLT4 T-cell leukemia	5 testicular cell suspensions with 10% MOLT-4 T-cells	Flow cytometry: leukemic cells in 5/5 testicular cell suspensions after sorting Xenotransplantation after sorting: tumor development in 3/3 mice grafted with 3 cell suspensions from the same animal
	FACS using singlet discrimination	Rhesus monkeys (n = 4)	Human MOLT4 T-cell leukemia	4 testicular cell suspensions with 10% MOLT-4 T-cells	Flow cytometry: leukemic cells in 1/4 testicular cell suspensions after sorting Xenotransplantation after sorting: tumor development in 0/4 mice
(Geens et al., 2011)	Culture of contaminated testicular cell suspensions by differential plating, followed by enrichment of CD49f+ germ cells with MACS. A selective matrix-adhesion (collagen I followed by laminin) based culture was applied to the previously enriched germ cells.	Human vasovasostomy patients (n = 5)	SB cells	10 testicular cell suspensions from 5 patients (P1, P2, P3, P4, P5) contaminated with 0% SB cells (controls) and: P1: 10% P2: 5% P3, P4, P5: 1%	Flow cytometry after purging: an average of 2.5% selected cells were SB-like cells (HLA-Class I+/CD49f—) PCR after purging: B-cell receptor in SB cells detected in 5/5 cell suspensions
(Dovey et al., 2013)	FACS with CD49e, HLA-ABC, CD45 malignant cell markers and EpCAM for germ cells <u>Protocol</u> : Selection of EpCAM—/HLA-ABC+/CD49e+ (putative MOLT-4 cells) and EpCAM+/HLA-ABC—/CD49e— (putative SSCs)	Human post-pubertal organ donors (n=NA)	Human MOLT4 T-cell leukemia	Testicular cell suspensions contaminated with 10% MOLT-4 T-cells. (n=NA)	Xenotransplantation of putative SSCs after sorting: 0% tumor development. Xenotransplantation of putative MOLT-4 cells after sorting: 23% tumor development when grafted in the intratubular space and 55% when grafted in the interstitial space
(Sadri-Ardekani et al., 2014)	Selective culture system <u>Protocol</u> : Culture of each patient's testicular cell suspension with one patient's leukemic cell suspension	Adult (n = 1) and prepubertal human testicular cells (n = 2)	Human B-cell ALL (n = 3)	15 testicular cell suspensions contaminated with 0% (controls), 0.04%, 0.4%, 4%, and 40% of leukemic cells from 3 patients	PCR for MRD after 20–26 days of culture: no expression of patient specific antigen receptor targets in all testicular cell suspensions

MHC, major histocompatibility complex; HLA, human leucocyte antigen; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; FACS, fluorescence activated cell sorting; MACS, magnetic activated cell sorting; EpCAM, epithelial cell adhesion molecule; PVG rats, Piebald-Virol-Glaxo rats; EL-4 mouse, mouse T-cell lymphoma cells; SB cells, human B-ALL cells; P, patient; SSC, spermatogonial stem cells; PCR, polymerase chain reaction; MRD, minimal residual disease; NA, not available.

Table V Cancer cell decontamination protocols for ovaries.

Study	Cell selection/ purging method	Ovarian cell source	Malignant cell source	Number of experiments	Outcome
(Soares <i>et al.</i> , 2015)	Mechanical purging by follicle pickup followed by 3 subsequent washes	Adult female ovarian tissue (n = 10)	BV-173 ALL cell line expressing BCR-ABL fusion transcript	9 ovarian cell suspensions	Fluorescence microscopy after purging: 1 leukemic cell among 772 follicles picked up and washed in 9 cell suspensions PCR after purging: no expression of the BCR/ABL fusion transcript in 9/9 cell suspensions
(Soares <i>et al.</i> , 2017)	Mechanical purging by follicle pickup followed by 3 subsequent washes	Cryopreserved ovarian tissue from adult (n = 10) and prepubertal (n = 2) leukemia patients	ALL (n = 6), AML (n = 5), CML (n = 1)	10 cell suspensions (2 excluded due to absence of molecular marker for PCR)	PCR after purging: performed in 9 cell suspensions. No expression of leukemia specific molecular markers in 9/9 cell suspensions (not performed in 2 patients due to absence of molecular marker and in 1 patient due to absence of monoclonal marker)
(Diaz-Garcia <i>et al.</i> , 2019)	Tissue incubation with dexamethasone	Cryopreserved ovarian tissue from leukemic prepubertal (n = 5) and post-pubertal (n = 1) patients (P1, P2, P3, P4, P5, P6) P1, P4, P6: 2 strips/patient P2: 8 strips P3: 4 strips P5: 4 strips	ALL (n = 6)	11 tissue fragments with prior dexamethasone incubation 11 tissue fragments without prior dexamethasone incubation (controls)	Xenotransplantation after purging: No leukemic cells at histology Expression of monoclonal rearrangements in P2, P3, P4. Xenotransplantation after purging: No leukemic cells at histology Expression of a monoclonal rearrangement in P5.
(Mulder <i>et al.</i> , 2019)	Tissue culture with oncogenesis inhibitor: YAP/TAZ in combination with imatinib	Adult female ovarian tissue (n = 8)	K562 CML	NA	Immunohistochemistry after sorting: absence of leukemic cells after 7 days of culture. PCR after purging: decreased expression of BCR-ABL fusion transcript
(Eijkenboom <i>et al.</i> , 2021)	Tissue culture with oncogenesis inhibitor: Aurora B/C kinases	Adult female ovarian tissue (n = 3)	Human CML cell lines: JURL-MK1, K562, MEG-01 (n = 3), human AML cell lines: KG-1a, NB4, NOMO-1 (n = 3) Primary AML cells (n = 4)	10 tissue fragments contaminated with each leukemic cell line	Histology after purging: Formation of syncytia in 5/6 cell lines and 4/4 primary AML cell lines. Persistence of tumor cells and no syncytia formed in MEG-01 contaminated tissue. Immunohistochemistry after purging: Anti-caspase 3 positive and Ki-67 negative cells in degraded syncytia of 5/6 cell lines and 4/4 primary AML cell lines. MEG-01 cell line negative for anti-active caspase 3 and Ki-67 positive.

NA, not available; BCR-ABL, breakpoint cluster region Abelson; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; PCR, polymerase chain reaction; P, patient; YAP/TAZ, Yes-associated protein/Transcriptional co-activator with PDZ-binding motif; KG-1a, Koefler and Golde-1a; NOMO-1, NODAL modulator-1.

inhibition of proteins directly involved in the tumorigenesis pathway. In a first study (Mulder et al., 2019), the YAP/TAZ oncogenesis pathway, that is found in leukemia, rhabdomyosarcoma, Ewing sarcoma, and breast cancer, was identified as the target for cancer cell purging. By culturing the contaminated tissue with Verteporfin to inhibit YAP/TAZ oncoproteins, efficient purging of ovarian tissue containing rhabdomyosarcoma-derived tumors was achieved. Using the same method with the addition of imatinib, the purging of leukemia cells was also successful. In both experiments no cancer cells were revealed by IHC after 7 days of culture. PCR also showed an absence of the PAX3-FOXO1 (a specific alveolar rhabdomyosarcoma translocation) fusion transcript and a decreased expression of the BCR-ABL fusion transcript. Based on dexamethasone's known apoptotic effect on leukemic cells, Diaz-Garcia et al. (2019) attempted purging ovarian tissue fragments from six patients with ALL. Based on a single initial ovarian biopsy per patient, only one patient had a positive PCR for cancer cell infiltration. Other tissue pieces were used to assess the decontamination protocol with half of each patient's samples used as control. Tissue fragments were incubated with dexamethasone or not before being xenotransplanted to SCID (severe combined immunodeficient) mice to check for tumor development. While tumor development was not described in any mice, PCR of the recovered grafts of three patients (including the patient who was positive at initial PCR) detected specific immunoglobulin or gene rearrangements in line with a failure of decontamination. Interestingly, when different ovarian fragments from the same patient that did not show tissue cancer infiltration at initial biopsy were xenotransplanted, PCR in some of the grafts showed the presence of cancer infiltration regardless of the use, or not, of dexamethasone.

Some studies addressed the possibility that such purging techniques could result in alteration of the follicles. Indeed, even though Peek's group (Mulder et al., 2019; Eijkenboom et al., 2021) has shown persistence of viability and intact morphology of the follicles, the assessment was limited to 24 h of culture, precluding any further functional evaluation. Moreover, as contamination of follicle suspensions was experimentally induced and although tumor formation within the ovarian tissue was described following the experimental injection of cancer cells in the Peek's group studies (Mulder et al., 2019; Eijkenboom et al., 2021), the question about the efficacy of the purging protocols for cancer cell invasion *in vivo* has not been addressed.

Another strategy is based on follicle isolation using follicle pickup followed, or not, by subsequent washing of the recovered pellets (Soares et al., 2015, 2017). In a preliminary study, ovarian tissue was harvested from women undergoing a laparoscopy for a benign gynecological disease and contaminated with a leukemic cell line (Soares et al., 2015). Using fluorescence microscopy and PCR, the authors confirmed that less leukemic cells were observed in all follicle suspensions when washes were performed after the follicle pickup with a micropipette (1 leukemic cell in 772 follicles with washes versus 196 leukemic cells among 499 follicles without washes) and that the BCR-ABL fusion transcript was only detected when no washes were performed. A cell viability assessment using calcein AM/ethidium homodimer as well as an evaluation of the morphology of the picked-up follicles by fluorescence microscopy after DAPI staining showed more than 92% well-preserved follicles. Using cryopreserved ovarian tissue obtained from six patients with leukemia and infiltration by leukemic cells proven through PCR, the previously reported Soares's protocol was able to

efficiently eliminate malignant cells, as demonstrated by PCR, with a detection threshold of as few as two leukemic cells (Soares et al., 2017). None of the studies performed further investigation of the morphology and functionality of the follicles obtained.

Discussion

The clinician's decision to use cryostored gonadal tissue from patients with cancer is often complicated by a lack of knowledge on both the presence or absence of cancer cells within the tissue and the success of techniques used to overcome the risk of cancer relapse when transplanting back reproductive material in the patient. Concerns are based on studies showing that the presence of only a few cancer cells in the gonadal tissue is sufficient to cause disease relapse (Hou et al., 2007a) and that leukemic cells have the capacity to migrate, as suggested when cells injected in one testis of a recipient rat were observed in the contralateral testis (Jahnukainen et al., 2001).

Auto-transplantation of cryostored prepubertal gonadal tissue is the main option for fertility restoration. Indeed, ovarian tissue autografting has already entered clinical practice for adult women (Practice Committee of the American Society for Reproductive Medicine. Electronic address: asm@asrm.org, 2019) and a few successful cases were also reported with pre- or peripubertal ovarian tissue (Demeestere et al., 2015; Matthews et al., 2018; Rodriguez-Wallberg et al., 2021). In males, tissue autografting also appears the most promising approach as offspring have been obtained in non-human primates after testicular tissue transplantation (Fayomi et al., 2019). However, when either the tissue fragments contain cancer cells or when no cancer cells are detected but the risk of focal cancer cell contamination is high, other options to restore fertility should be considered, as shown in Fig. 1, including the perspective of generating germ cells from extra-gonadal sources (Yamashiro et al., 2018).

Knowledge of the actual rate of gonadal tissue infiltration by cancer cells is crucial for a risk assessment before transplantation, and the development of effective decontamination techniques is consequently in the forefront of research.

In this review, we therefore first focused on reported rates of cancer cell contamination in testes and ovaries from prepubertal patients suffering from either leukemia or lymphoma. A larger number of studies were found on the prepubertal testis as it was long considered a preferential site for leukemia and lymphoma relapse and assessing testis contamination was therefore critical for patients in remission.

A limited number of authors investigated this matter in prepubertal girls, and it is also of note that the assignment to a prepubertal state or not based on age as a proxy-indicator is a limitation of the available data on prepubertal girls.

While overall contamination rates were at 37% for the testis and 12% for the ovary, the ranges appeared to be large (for testes: 21–100% in leukemia and 15–50% in lymphoma; for ovaries 0–66% in leukemia and 0% with one case reported of a contaminated ovary in lymphoma) (Tables I and II). Several factors were suggested to account for such large ranges of cancer cell contamination rates, including sampling techniques (whole gonad, wedge biopsy, fine needle aspiration cytology), detection methods (histology, IHC, PCR, xenotransplantation), and the disease status of patients at the time of evaluation.

Putting these figures in the context of FP management and care pathways is crucial. In clinical routine, testicular and ovarian tissue are preferentially harvested prior to chemo-radiotherapy to avoid their deleterious impact on germ cell number and function (Wallace *et al.*, 2005; Stukenborg and Wyns, 2020). However, as there is currently no reliable and validated technique to purge gonadal tissue of cancer cells, the timing of gonadal sampling and banking is a matter of debate. On the male side, there is no consensus about the optimal timing of ITT banking. While cryopreservation is preferably performed prior to any gonadotoxic treatment because of the known deleterious effect of chemo-radiotherapy on spermatogonia (Wyns *et al.*, 2010), some teams will now bank tissue after a few rounds of chemotherapy (Ho *et al.*, 2017; Braye *et al.*, 2019; Valli-Pulaski *et al.*, 2019; Goossens *et al.*, 2020) but the actual impact will only be known when data from further experimental studies assessing the quality of gametes derived from stored tissue become available. Similarly, for ovarian tissue cryopreservation, several teams have taken the option of harvesting after one or two rounds of chemotherapy as it did not increase either the time to ovarian function recovery (defined as the time between tissue transplantation and the occurrence of menstruation) (Poirot *et al.*, 2019) or the pregnancy rate (Dolmans *et al.*, 2021). However, lower graft survival rates and a lower cumulative incidence of pregnancy in patients that were given alkylating agents prior to ovarian tissue cryopreservation compared to those who had received other chemotherapy protocols (e.g. ABVD: adriamycin-bleomycin-vinblastine-dacarbazine) was observed (Dolmans *et al.*, 2021). The true impact of alkylating and non-alkylating drugs on ovarian tissue should nevertheless be further investigated in studies that include patients receiving similar numbers and sizes of grafts as well as tissue pieces with comparable follicular densities, as all are independent factors for the recovery of ovarian function after transplantation (Poirot *et al.*, 2019).

In this regard, recent guidelines from ESHRE (ESHRE Guideline Group on Female Fertility Preservation *et al.*, 2020) and the European Society for Medical Oncology (Lambertini *et al.*, 2020) also suggested that postponing ovarian tissue cryopreservation after a few rounds of chemotherapy could help to diminish the number of cancer cells, particularly in patients with leukemia.

The reliability of reported cancer cell contamination rates is another concern when considering future use of gonadal tissue. Indeed, a high number of studies are old and performed with detection techniques, such as histology and IHC, that have known limitations while the accuracy of available techniques has evolved. The use of PCR for specific markers of the disease has helped detect what is known as MRD, which in the clinical setting is residual cancer cells following treatment for a hematological disease (Kruse *et al.*, 2020). It has been suggested that the risk of MRD in the ovary is correlated to the MRD status in the patient's bone marrow (Jahnukainen *et al.*, 2013) as well as to the blood lymphocyte count (Soares *et al.*, 2017). However, on the male side, such correlations were not established. Hence, defining the minimum thresholds for risk of relapse remains a challenge, stressing the importance of developing robust decontamination techniques that enable complete elimination of cancer cells while preserving concomitant viability and functionality of the germ cells.

Regarding the testis, FACS was the most frequently used cell sorting method but has not shown very promising results in obtaining cancer cell-free preparations with human samples. Moreover, besides the need for effective and efficient cell selection, assessment of viability,

proliferation, and differentiation potential of sorted SSCs was not performed on human cells. However, it is noticeable that lower cell viability was observed after sorting murine cells (Geens *et al.*, 2007). In the future, decontamination protocols for testicular tissue or cells that have proven successful with animal samples should be further investigated using human tissue, but the main obstacle is the scarcity of human prepubertal tissue available for research purposes. With regards to ovaries, innovative techniques have been developed to decontaminate the tissue. These are based on the use of apoptotic effects on leukemic cells or oncogenesis inhibiting factors that directly target malignant cells and spare the ovarian microenvironment. Besides the need to confirm the efficacy of the latter, agents interfering with the YAP/TAZ and Aurora kinase pathways have yet to be assessed for safety with regards to fetal development and any resulting effect on offspring. Efforts might also be directed towards phototherapy that could target the selective killing of cancer cells (Moghassemi *et al.*, 2022).

Until decontamination protocols are shown to be reproducible and further investigations on follicle functionality are carried out, a case-by-case evaluation of the safety of the banked tissue remains the only option for fertility restoration. In this regard, successful auto-transplantation of cryopreserved ovarian tissue from patients with lymphoma (Donnez *et al.*, 2004; Oktay and Oktem, 2010) and leukemia (Meirow *et al.*, 2016; Shapira *et al.*, 2018; Silber *et al.*, 2018) without relapse of the cancerous disease were reported.

Furthermore, the real value of decontamination methods is questionable as most protocols have been developed with artificially contaminated cell suspensions and not with gonadal tissue harvested from patients with cancer, i.e. with *in vivo* invasion by cancer cells.

The question also remains of how far the evaluation of only part of the available tissue is sufficient to confidently transplant it back to a patient who has recovered, since the part of the tissue that is evaluated is different from that used for transplantation, especially as opposing results have been observed among fragments from the same patient (Diaz-Garcia *et al.*, 2019).

Conclusion

At the present time, there are no accurate or reproducible protocols to detect and eliminate cancer cells in harvested gonadal tissue despite many advances in recent years. The figures summarized in this review may help the clinician to estimate risks related to the fertility restoration methods available. Unless research on either decontamination protocols or on strategies other than auto-transplantation rapidly evolve, clinicians will often face the difficult decision of whether to use cryopreserved gonadal tissue based on a case-by-case evaluation of the risks and benefits. However, they should not refrain from cryopreservation of prepubertal gonadal tissue as patients that are young children at the time of harvest may in 10–20 years benefit from ever-evolving fertility restoration strategies, whether in the field of purging cancer cells or cell isolation and selection aimed at auto-transplantation, as well as cell and tissue maturation *in vitro*.

Data availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Authors' roles

D.K. performed the literature search and drafted the manuscript. D.K. and C.W. ideated the paper outline. M.K. and C.A.A. contributed to the literature search. C.W., M.K., and C.A.A. critically reviewed the manuscript. C.W. approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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