# ORIGINAL ARTICLE

# Fertility preservation issues in pediatric hematopoietic stem cell transplantation: practical approaches from the consensus of the Pediatric Diseases Working Party of the EBMT and the International BFM Study Group

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Fertility preservation is an urgent challenge in the transplant setting. A panel of transplanters and fertility specialists within the Pediatric Diseases Working Party of the European Society for Blood and Marrow Transplantation (EBMT) and the International BFM Study Group provides specific guidelines. Patients and families should be informed of possible gender- and age-specific cryopreservation strategies that should be tailored according to the underlying disease, clinical condition and previous exposure to chemotherapy. Semen collection should be routinely offered to all postpubertal boys at the diagnosis of any disease requiring therapy that could potentially impair fertility. Testicular tissue collection might be offered to postpubertal boys; nevertheless, its use has been unsuccessful to date. Oocyte collection after hormonal hyperstimulation should be offered to postpubertal girls facing gonadotoxic therapies that could be delayed for the 2 weeks required for the procedure. Ovarian tissue collection could be offered to pre-/post-pubertal girls. Pregnancies have been reported after postpubertal ovarian tissue reimplantation; however, to date, no pregnancy has been reported after the reimplantation of prepubertal ovarian tissue or in vitro maturation of pre-/post-pubertal ovarian tissue. Possible future advances in reproductive medicine could change this scenario. Health authorities should prioritize fertility preservation projects in pediatric transplantation to improve patient care and quality of life.

## INTRODUCTION

During the Pediatric Diseases Working Party (PD WP) of the 42nd Meeting of the European Society for Blood and Marrow Transplantation (EBMT), we learnt that 56 000 transplantations in children and adolescents had been reported to the EBMT Registry between January 2000 and January 2016. This translates into ~ 3500 transplantations per year (unpublished data) or an average of 10 children or adolescents transplanted in Europe every day.

According to the report of the PD WP, 29% of the patients received autologous grafts, and 71% received allogeneic grafts. Virtually all the patients in the former group received transplants for malignancy, whereas among the latter group, 70% were transplanted for malignancies and 30% for nonmalignant diseases (unpublished data). The proportion of patients undergoing transplantation for nonmalignant versus malignant diseases has increased over time, from 36% in 2002 (unpublished data) to 46% in 2012.[1]

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According to the pediatric EBMT Registry, two-thirds of all patients who receive transplants during childhood or adolescence are alive and therefore at risk of late effects, of which infertility is the most frequent complication.[2]

The gonadotoxic effects of chemotherapy and radiotherapy are well known.[2–4] The majority of conditioning regimens cause germ cell loss in the ovaries and testes, accelerating the end of the reproductive lifespan in females and impairing spermatogenesis in males.[5–7]

No gonadal protection can be considered foolproof in patients for whom the conditioning regimen includes TBI, although ovarian shielding has been proposed.[8]

The risk of infertility varies, and fertility may be incrementally reduced to the level of permanent sterility depending on the underlying disease, type and dosage of chemotherapy given before transplantation, conditioning regimens and the recipient's age at the time of transplantation.[3,9] Furthermore, the ovarian reserve before treatment is critical for girls.[5]

Overall, <5% of transplanted individuals report having children after transplantation, but data are sparse for adults who were transplanted during childhood or adolescence.[3,4,9] In 1996, Sanders et al.[10] reported offspring in only 5% of 708 long-term survivors who had been transplanted during either childhood or adulthood after conditioning with cyclophosphamide alone or in combination with either busulfan or TBI. Pregnancies mainly occurred after regimens consisting of cyclophosphamide alone, as typically used in cases of severe aplastic anemia, in >50% of female patients and 25% of male patients' partners.[10] Pregnancies were reported very rarely after busulfan-based conditionings (<1% of women and 7% of men) and TBI-based conditionings (1% overall, regardless of gender), with single-dose TBI impairing gonadal function more than fractionated or hyperfractionated TBI.[9,10] A number of investigators reported a higher probability of residual fertility when the transplant occurred at a young age, at least in females.[3,5,9,10]

These retrospective reports did not consider whether couples were trying to conceive. Recently, Dyer et al.[11] reported a cross-sectional study assessing sexual health and fertility in 421 adult survivors following allogeneic transplantation; overall, only 3% of them had offspring after transplantation, whereas 22% reported that they were trying to conceive.

Borgmann et al.[9] investigated fertility in a cohort of 344 patients with a median age of 19 years (range 12–35) at a median of 6 years post allogeneic transplantation that was performed during childhood or adolescence at 7 centers in Europe between 2000 and 2005. Fertility impairment was diagnosed in 69% of the male participants and 83% of the female participants. Seven children had been born in total, five of them to three patients with severe aplastic anemia who were grafted after a conditioning regimen consisting of cyclophosphamide alone; no offspring were reported in the 148 patients conditioned with TBI, and only 1 was reported among the 100 patients conditioned with busulfan.[9]

Vatanen et al.[12] evaluated that out of 92 young women with a median age of 22 years who were transplanted at a median age of 13 years, 10 gave birth to 11 children. Five of the 10 mothers had received a TBI-containing conditioning.[12]

Conditioning regimens in pediatrics are mainly myeloablative (87%), but the proportion of children receiving nonmyeloablative or reduced-intensity/toxicity conditioning regimens has increased from 8% in 2000 to 16% in 2015 (unpublished data). The extent of the lower gonadotoxic impact of reduced-intensity conditioning remains to be assessed.[13,14] Two pregnancies have been recently reported within the first 2 years after transplantation in a woman transplanted at 19 years of age after reduced-intensity conditioning.[15]

The large number of patients transplanted as children or adolescents who become long-term survivors has progressively increased awareness of the impact of infertility on quality of life. Fertility and sexual health impairment will deeply affect the quality of life of most transplanted patients, at least those who have received myeloablative conditioning and are otherwise considered 'successfully cured' by transplantation. Recent advances in reproductive medicine make fertility preservation a burning challenge and have led the EBMT PD WP to establish a task force focused on fertility preservation issues.[16–21]

## CONSENSUS

A panel of fertility specialists within the EBMT PD WP from 15 countries met in Baden in September 2015 and continued their discussion for the following months and extended it within the Stem Cell Transplant Committee of the International BFM Study Group with the aim of examining fertility preservation issues and producing guidelines.

A wide variety of approaches among countries emerged because of a combination of regulatory, scientific, financial and cultural issues.

There was consensus on the following issues:

A. specifically trained team, including a specialist in reproductive medicine and a pediatric oncologist/hematologist/transplanter, should be available at all transplantation centers to provide age-appropriate counseling to the recipient (child, adolescent, young adult) and the family.

B. An accurate history (pubertal status, menstrual history, ability to have an erection and ejaculate, sexual activity), physical examination (Tanner stage, testis volume), hormonal levels (follicle-stimulating hormone, luteinizing hormone, human chorionic gonadotropin, anti-Müllerian hormone (AMH)) and assessment of the patient's attitude should precede the counseling.

C. All patients and their parents should receive information on:

  - the risk of infertility, estimated according to age at transplant, previous chemotherapy and/or radiation, conditioning intensity (TBI, busulfan, alkylating agents);
  - available preservation techniques that, other than sperm preservation, may be considered novel in postpubertal girls and experimental in prepubertal children;
  - specific timelines for each technique that may not be compatible with the treatment schedule required to control the underlying disease.

D. Fertility preservation options should be discussed, offered and tailored according to several factors:.

  - fertility status before transplantation, specifically in patients with malignancies already treated with chemotherapy and radiotherapy;
  - patient performance scores and clinical condition (neutropenia, thrombocytopenia, infections) that may limit the feasibility of surgery;
  - prognosis, wishes, expectations, culture, ethical issues, logistics and timing that should all be considered and carefully assessed.

E. Informed consent must be obtained in writing from patients and/or parents after they have received appropriate information on procedures, associated risks and, in some countries, costs.

Specific guidelines according to pubertal status and gender were agreed upon.

A flowchart is shown in Figure 1 that details fertility preservation strategies for children, adolescents and young adults upon referral for transplantation.

## BOYS

### Postpubertal boys

*Semen cryopreservation.* Semen collection and cryopreservation carries no risks and is an established fertility preservation method that is appropriate for postpubertal boys. It should be offered to all postpubertal males at any risk of fertility impairment.[22–24]

Semen collection is likely to be feasible in pubertal boys with at least a Tanner III stage and a testicular volume of >6 ml.[23–25] Younger postpubertal boys who are unable to ejaculate may
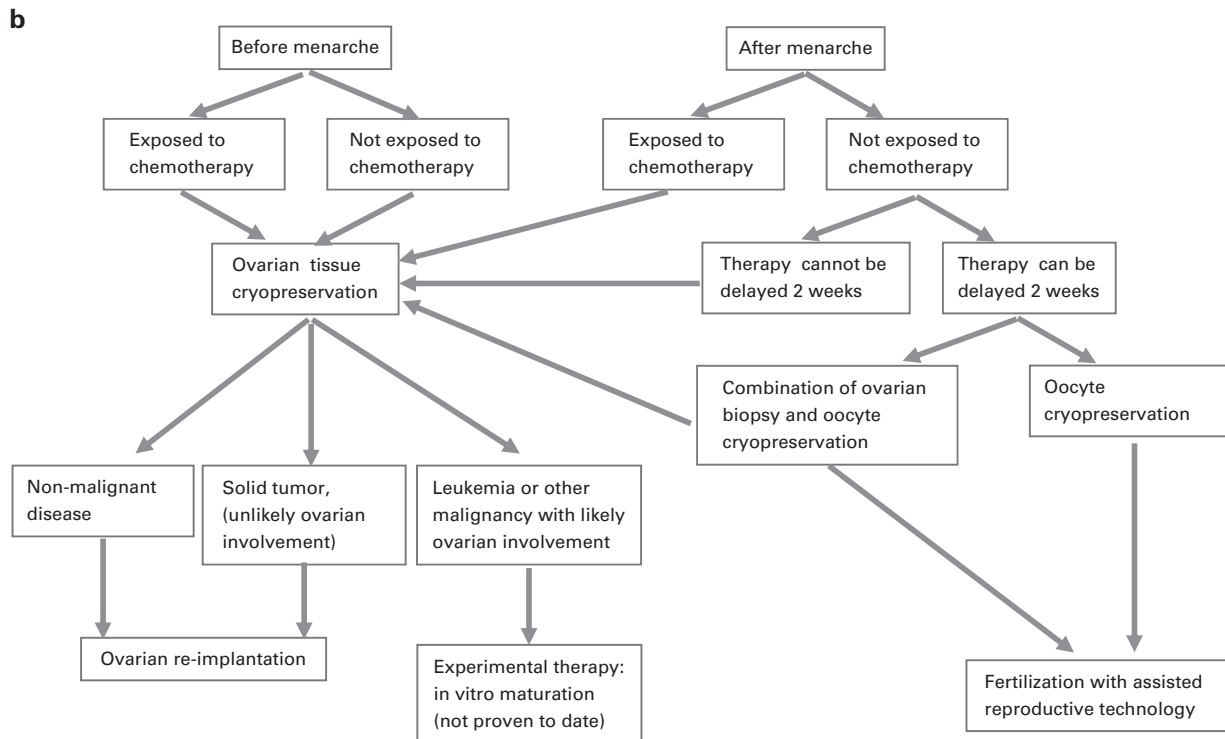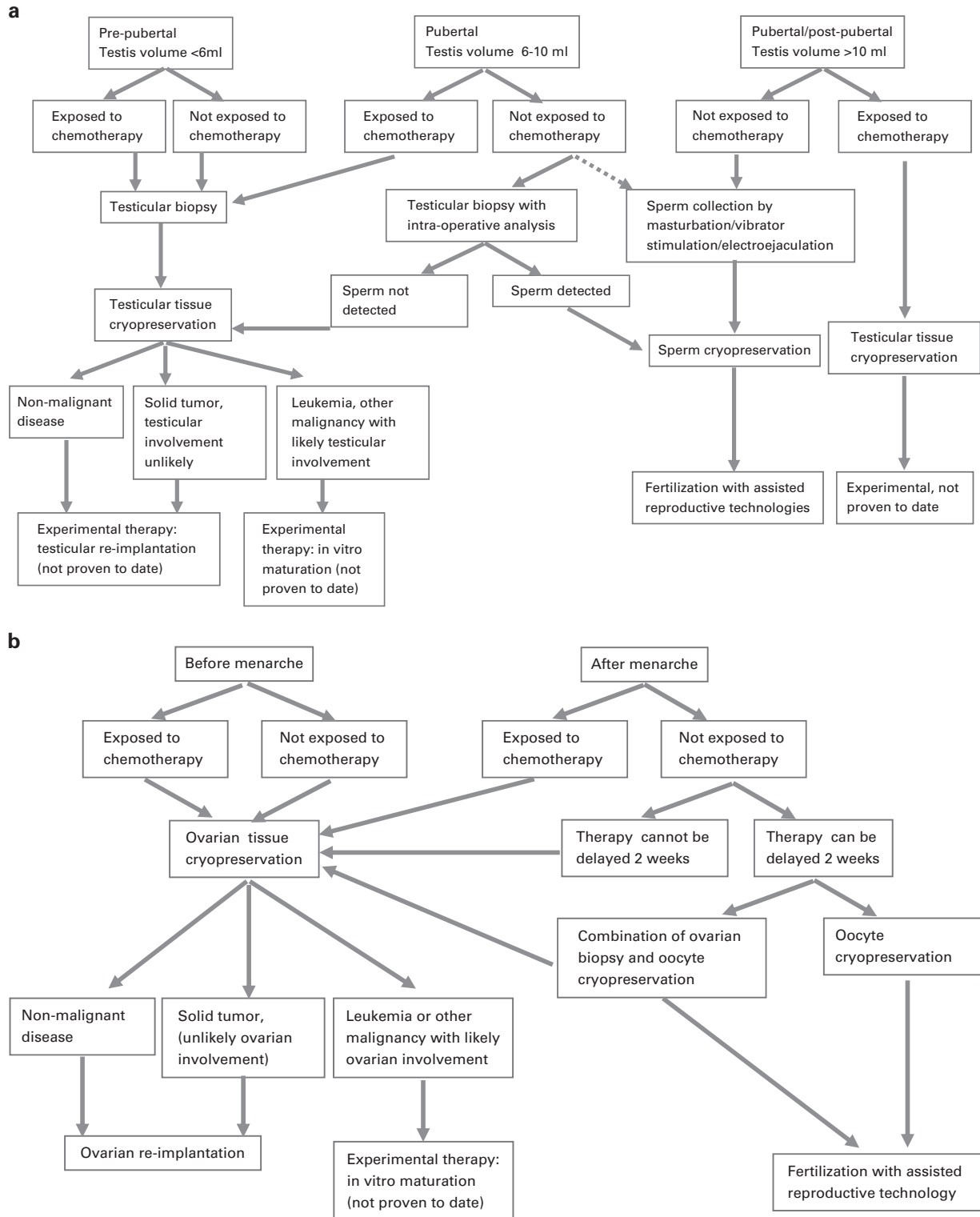
**a**

**b**

**Figure 1.** Algorithm for fertility preservation strategies for boys (**a**) and girls (**b**) with no previous gamete or gonadal tissue cryopreservation upon referral for transplantation. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

be offered vibrator stimulation or electroejaculation under sedation.[23,26]

If a postpubertal patient is unable to produce an ejaculate or has azoospermia, an invasive procedure under general sedation may be considered to retrieve testicular sperm.[24]

Sperm banking is a routine procedure in fertility preservation; nevertheless, the use of the stored sperm requires assisted reproductive technology (*in vitro* fertilization, intrauterine insemination) to achieve pregnancy, and success depends on sample quality and quantity.[24] The availability of more than one sample, collected at an interval of a few days, generally increases the chances of success.[25–27]

In cases of malignant disease, semen collection should be organized at diagnosis, before chemotherapy can jeopardize cellularity and viability and cause a reduction of sperm counts, as differentiating spermatogonia are the most sensitive to gonadotoxic injuries.[27,29]

Once chemotherapy has started, testicular involution progressively leads to virtually invariable azoospermia within 3 months.[7,24] Azoospermic collections should be avoided and are frustrating for patients and parents. Moreover, the quality of sperm that is produced even a few weeks after the initiation of chemotherapy is not appropriate for cryopreservation, as chemotherapy may induce DNA breakages and chromosomal abnormalities in spermatozoa.[29] Therefore, semen cryopreservation during the testicular involution post chemotherapy should not be carried out.[29]

Recovery from oligo- or azoospermia is variable and depends on the extent of both spermatogonial stem cell destruction and impairment of the somatic environment that normally supports their differentiation.[29] In the event of spermatogenetic recovery, sperm DNA breakages and low chromatin compaction have been reported up to 24 months post chemotherapy.[29]

Leukemia is the most common pediatric cancer and represented 30% of all the indications for transplantation in pediatrics in 2012.[1] Only patients with relapsed disease or at very high risk of relapse are considered for transplantation; the latter group is often determined by the response to the first few months of treatment. Therefore, these patients may be heavily pretreated or at best have had 1 to 3 months of chemotherapy. Steroid treatment alone, if given before chemotherapy in acute lymphoblastic leukemia, is not gonadotoxic *per se* but might limit a boy's ability to ejaculate.[23,24]

Sperm collection should be arranged before chemotherapy starts and not several months later, when eligibility for transplantation is determined. At the time of referral for transplantation post chemotherapy, therefore, it may be too late to plan for sperm collection and testicular tissue might be collected and cryopreserved, usually within specific experimental protocols.

Sperm collection in all newly diagnosed patients who might require transplantation in the future would overcome this problem but would mean that many boys would never use the stored sperm.

Postpubertal males represent between 5 and 30% of the population of an 'average' pediatric and adolescent hematology-oncology center depending on the center's disease profile (that is, leukemias versus lymphomas, age range). According to current chemotherapy protocols, ~ 10% of children newly diagnosed with acute lymphoblastic leukemia and > 50% of those who relapse, 10% of those with lymphoma, <50% of those with acute myeloid leukemia and the majority with myelodysplasia are considered eligible for transplantation. If sperm was collected from all postpubertal boys, only ⩽ 25% of the samples might be used in the future.[21,23]

### Prepubertal boys

*Spermatogonial stem cell preservation.* Spermatogenesis has not begun in immature boys, and hence the collection of testicular tissue containing spermatogonial stem cells is the only feasible approach for prepubertal boys.[7,24,28,29]

In addition, postpubertal patients who are azoospermic may be considered for testicular tissue collection.

In peripubertal boys with a testis volume of 3–6 mL, spermatogenesis may have started. These boys might be offered biopsies to check for possible intratesticular sperm that should be collected and cryopreserved using specific protocols aimed at preserving mature sperm cells.[24,25]

In cryopreserved samples, spermatogonial maturation up to mature spermatozoa can be generated in non-human primate models by using germ cell transplantation and testicular tissue reimplantation; however, this technique has not been successful in humans, and no conceptions have been reported to date in humans using these approaches.[30–32]

Testicular tissue banking should be considered highly experimental. It is not a routine procedure, but it can be offered, usually as part of a specific research protocol.[3]

The ideal time for testicular biopsy is before starting gonadotoxic chemotherapy, which will destroy spermatogonial cells, predominantly the more mature ones.[24,29] Nevertheless, given the experimental nature of this relatively invasive procedure, testicular tissue banking should not be recommended at the onset of a disease that requires treatment with chemotherapy but has a relatively low risk of infertility; instead, tissue banking should occur as soon as transplantation eligibility is recognized. The potential testicular effect of any previous chemotherapy exposure must be accepted when considering any type of storage for fertility preservation in pretreated patients.

Cryopreservation should be considered for any gonadal tissue removed for diagnostic or therapeutic purposes. When orchidectomy is performed for leukemic testicular relapse, tissue cryopreservation may be considered highly experimental, but *in vitro* spermatogonial maturation may become feasible in the future. Leukemic contamination does not affect the number of spermatogonia in the testicular tissue that could be cryopreserved regardless of the extent of infiltration, either molecular or morphological.[33]

## GIRLS

### Postpubertal girls

*Oocyte cryopreservation.* Transvaginal oocyte collection after hormonal ovarian hyperstimulation, followed by cryopreservation, is an established technique in adult females.[17,34–36] On average, at least 14 days are required for the process.[36–38] Cryopreserved oocytes would remain available for assisted reproductive techniques.

The absence of previous sexual intercourse in girls may contraindicate transvaginal access, although it should not be considered an absolute contraindication. The possibility of proceeding should be extensively discussed with the girl and her family, taking into account any specific cultural issues.

Ovarian stimulation and oocyte collection is a relatively invasive procedure compared with sperm collection; therefore, it is not generally recommended at the diagnosis of diseases for which treatment carries a relatively low risk of infertility but should be planned as soon as eligibility for transplantation is apparent.

In cases of malignancy requiring chemotherapy, oocyte stimulation and collection should be performed before any antineoplastic drug is initiated.[34] Chemotherapy will cause germ cell loss, predominantly of the most mature follicles, which are the follicles that will produce oocytes after stimulation, in contrast to the more primordial residual follicles.[18–20,34,35] Moreover, oocyte

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collection during or shortly after chemotherapy is contraindicated because of the risks of chromosomal aberrations, as shown in mice treated with alkylating agents.[19]

The timing of the procedure may not fit with the need to control the underlying disease, especially leukemia, for which the initiation of chemotherapy cannot be usually delayed.[34,35,39]

Moreover, as oocyte aspiration requires an invasive procedure with a transvaginally inserted needle, the risk of infection and bleeding should be carefully evaluated in pancytopenic patients as a severe pelvic infection could lead to the postponement of chemotherapy or the transplantation itself.

Thrombotic risk associated with hormonal therapy should be evaluated, particularly in patients at higher risk because of their underlying disease, such as those with sickle cell disease or thrombophilia.

*Embryo cryopreservation.* Oocyte collection directly followed by fertilization and subsequent embryo cryopreservation is rarely an option in pediatric patients because of the lack of a committed sexual partner.[18,20]

*Ovarian suppression with GnRHa.* Gonadal function is better preserved in patients transplanted during childhood than after puberty.[3,5,9,10] This observation suggests that ovarian suppression might reduce chemotherapy-induced gonadotoxicity. Alternatively, the presence of a higher number of ovarian follicles in prepubertal girls compared with older girls might explain this difference.

Ovarian suppression in post-pubertal girls by means of gonadotropin-releasing hormone agonists (GnRHa) during chemotherapy may increase the chance of restoring menstrual function and eventually reducing the risk of premature ovarian failure, but no conclusive data have been reported.[40–44] GnRHa during chemotherapy will reduce menstrual bleeding that might justify its use *per se.*

Most available data are derived from adult women, mainly those with breast cancer.[41–43]

Demeestere *et al.*[44] recently reported a 5-year update on a cohort of 129 women with lymphoma who were randomly assigned to receive norethisterone alone or with triprorelin as a GnRHa. No statistically significant difference was found in the incidence of premature ovarian failure, evaluated by follicle-stimulating hormone and AMH levels, or pregnancies in the two groups. Hematopoietic stem cell transplantation was associated with a 68-fold increase in the relative risk of ovarian failure (*P*-value 0.002).[44]

The efficacy of GnRHa-mediated ovarian suppression during chemotherapy is still controversial, and no data are available in the pediatric transplant setting.

*Ovarian tissue cryopreservation.* The collection of mature oocytes and embryos requires at least a 2-week period, but ovarian tissue may be collected on short notice, ideally before treatment initiation. After cryopreservation, it will remain available for reimplantation.[45]

Ovarian collection is usually performed laparoscopically; laparotomic access is used when abdominal surgery is required for other purposes.[16,18,20,46] Postpubertal ovarian collection is usually considered when oocytes cannot be harvested, often because of the restricted time schedule.[18,20,45]

Half an ovary or at least 5 cortical biopsies should be harvested in postpubertal girls.[20,46] The greater the risk of infertility, the larger the amount of ovarian tissue that should be harvested; the potential reduction in ovarian function *per se* must be carefully considered in cases of nonmyeloablative conditionings that may be associated with residual fertility.[3,12,14]

*Combined oocyte and ovarian collection and cryopreservation.* Ovarian biopsies can also be followed by ovarian hyperstimulation to increase the proportion of successful collections.[47,48]

Cryopreservation of *in vitro* matured oocytes in addition to ovarian tissue is also possible. At least three live births have been reported following this technique, and this is a relevant addition in cases of leukemia, in which it is crucial to avoid blast dissemination.[49]

### Prepubertal girls

*Ovarian tissue cryopreservation.* Ovarian tissue collection followed by cryopreservation is the only feasible approach to fertility preservation in prepubertal girls.[50–52]

A whole ovary should be removed in prepubertal girls because of the small size of the gonad.[50]

Prepubertal ovarian tissue maturation is experimental as the *in vitro* growth of primordial follicles to mature oocytes has not yet been achieved in humans.[52] No pregnancies have been reported in humans so far in the scientific literature, neither after prepubertal ovarian tissue reimplantation nor after *in vitro* maturation. A single case was reported in the general press only in the United Kingdom, but no scientific report was available at the time of this submission.

The first xenografting experiments have shown promising results, with preantral follicles from prepubertal patients successfully developing after reimplantation.[53]

In contrast, *in vitro* studies have shown very little follicular development of prepubertal tissue to secondary stages and a high proportion of abnormal, nongrowing follicles.[19,54]

Similarly, histological analyses suggest that the diameters of oocytes and primordial and transitory follicles progressively increase with female age from 4 to 30 years.[19]

Differences in the rate of follicle activation and growth *in vitro* may indicate intrinsic differences in follicular developmental capacity between young girls and adults.[54,55]

Ovarian tissue collection during chemotherapy is still experimental as no data exist regarding the success and the safety of this procedure. The *in vitro* studies suggest that exposure to progressively increasing cumulative doses of alkylating agents before ovarian cryopreservation may decrease the survival of cultured human ovarian follicles.[19]

### Issues in females

*Ovarian tissue reimplantation.* Ovarian tissue can be reimplanted into the pelvic cavity at any time after transplantation to restore ovarian function and allow natural conception.[56–60]

At present, almost 100 healthy children have been born after the reimplantation of adult ovarian tissue after chemotherapy treatment for adult cancer.[56–60] A pregnancy rate of 33% and a delivery rate of 25% after ovarian tissue reimplantation were reported by the FertiPROTEKT, a network covering German-speaking countries, that offers updated consultations and interventions regarding fertility to women and men facing chemotherapy or radiotherapy.[59]

Most of the available data come from women who were treated for solid tumors, particularly breast cancer. The results of a smaller series of patients who underwent hematopoietic stem cell transplantation have also been reported. All successful reimplantations refer to ovarian tissue collected from postpubertal females.[56–60]

In 2015, one healthy offspring was reported after hematopoietic transplantation as a result of the natural fertilization of reimplanted ovarian tissue collected at 13 years of age. The girl had undergone transplantation to treat a nonmalignant hematological disease and at the time of ovarian collection was premenarchal, with gonadotropin levels consistent with primary amenorrhea.[61]

*Ovarian tissue in malignancy.* Two-thirds of children and adolescents reported to the EBMT Registry underwent transplantation for malignant diseases that carry the risk of contamination of gonadal tissue.[1,62,63] Extensive discussion with the patient regarding the risk of dissemination in the event of reimplantation would allow individuals to make an informed and conscious decision. The risk should be considered low in cases of solid tumors and Hodgkin lymphomas, intermediate in cases of non-Hodgkin lymphomas and highest in cases of neuroblastoma and leukemia, primarily acute lymphoblastic leukemia, the most frequent indication for allogeneic transplantation in children.[1,18,62,63]

Although solid tumors are considered to carry a low risk of contamination, ovarian dissemination cannot be ruled out. As previously reported, in one out of seven patients affected with Ewing sarcoma, disease molecular translocation was detected in the ovary despite normal histology.[64]

Ovarian tissue reimplantation without relapse was reported in one patient with chronic myeloid leukemia and one patient with acute myeloid leukemia.[58,63]

Nevertheless, despite the lack of pathological/immunohistochemical evidence, the disease can be detected in the ovary using molecular techniques, as previously reported.[65]

A recent study of 18 patients with leukemia (CML or ALL) showed that leukemic tumors occurred in 4 cases after thawed human ovarian cortical tissue was xenografted into mice.[62]

The danger of reseeding cancer with ovarian grafting, especially in patients with leukemia (mostly ALL and CML), should be considered a contraindication for reimplantation. Reimplantation in leukemia should be avoided, even when minimal residual disease cannot be detected in the stored product with molecular techniques that are not validated for such a purpose.

Upon ovarian tissue collection, possibly available oocytes may be harvested and directly fertilized for embryo cryopreservation as long as they are fresh. At present, no fertilization technique allows the *in vitro* maturation and fertilization of oocytes from thawed ovarian tissue or thawed oocytes harvested from surgically removed ovarian tissue.

Nevertheless, both prepubertal and postpubertal gonadal collection from patients with leukemia can be considered, in view of future developments, for *in vitro* maturation and subsequent *in vitro* fertilization.

*Timing of collection and chemotherapy.* Disease features at presentation might require early initiation of chemotherapy to control the disease. Treatment schedules may leave no room for fertility preservation strategies.

Nevertheless, the launching of a designated fertility preservation program is expected to yield significantly earlier referrals and increase the number of collections performed for fertility preservation.

As reported by Ben-Aharon *et al.*,[66] out of 62 female patients (median age 13 years, range 18 months to 18 years) referred in Israel over a 36-month period, 41 underwent ovarian cryopreservation, and 11 underwent oocyte cryopreservation, with only 6 declines because of parental decisions. Even more remarkably, half of the patients underwent collection before chemotherapy initiation, and one-fourth were affected with leukemia. As expected, the mean number of mature oocytes eventually vitrified was significantly higher in the chemotherapy-naive patients compared with the chemotherapy-exposed patients (mean 12 oocytes (range: 1–42/patient) versus 2 (range: 0–7/patient)).[66]

*Advances in in vitro oocyte maturation.* Abir *et al.*[49] reported a protocol that combined *in vitro* maturation of germinal vesicle-stage oocytes followed by vitrification with the freezing of cortical ovarian tissue for both chemotherapy-naive pediatric patients and patients after the initiation of cancer therapy. Immature oocytes could be collected from cortical ovarian tissue, usually after oophorectomy, even in very young girls, without hormonal stimulation and then matured *in vitro* and vitrified. In a series of 42 girls ranging from 1 to 18 years, ovarian tissue could be successfully collected from 78% of them. Oocytes were obtained from 20 patients before chemotherapy and from 13 after chemotherapy initiation.[49] Although more oocytes were collected and matured from chemotherapy-naive pediatric patients, ovarian tissue and immature oocytes were also retrieved from young girls for whom cancer therapy had already been initiated.[49] Vitrified *in vitro* matured oocytes might serve as an important gamete source for pediatric female patients, although further studies are needed on the fertility-restoring potential of oocytes from pediatric and prepubertal patients, especially after exposure to chemotherapy.[49]

*Puberty induction.* Puberty may be induced by the reimplantation of ovarian tissue in girls with premature primary ovarian failure due to gonadotoxic therapy, a form of hypergonadotropic hypogonadism.[67,68]

However, upon ovarian reimplantation, the elevated gonadotrophins could cause the sudden growth of several follicles, leading to a sharp increase in serum estrogen levels and regular ovulation. This might lead to accelerated pubertal development that is associated with a number of disadvantages, including premature cessation of growth.[69]

In general, gonadal tissue should be reserved for reproductive purposes; hormonal replacement therapy *per se* may not justify gonadal reimplantation and could be contraindicated in malignancy cases.

## POST TRANSPLANTATION MONITORING

Fertility assessment is crucial after transplantation, particularly for patients who were not offered or could not access any fertility preservation strategy before transplantation.[2,5–7,10]

It is important to recognize that the exhaustion of the ovarian follicle pool in females and spermatogenetic recovery from proliferating spermatogonia in males are dynamic processes and that the timing of fertility assessment after transplant is important.[6]

Recovery from fertility impairment has been observed between a few months and 12 years post transplantation in almost 5% of 361 patients who underwent transplantation during childhood or adolescence. This occurred at a median of 4 years after transplantation in females and 2 years in males, as described in a recent report by Pfizer *et al.*[6]

Rovó *et al.*[70] reported the results of semen analysis at a median follow-up of 9 years after transplantation in 39 long-term survivors transplanted at a median age of 25 years. Out of 19 patients, 10 (53%) aged ⩽ 25 years had some documented sperm production.

The Tanner stage should be assessed every 6 months in pre- and peri-pubertal patients. Periodical monitoring of hormonal levels and pubertal growth, plus menstrual history and pelvic ultrasound in women and testicular volume and semen analysis in males, are important in assessing fertility.[6,70]

An adult testicular volume of ⩾ 15 ml after pediatric transplantation predicts the presence of spermatozoa in the seminal fluid with 80% sensitivity and 91% specificity.[71]

According to recent international guidelines for gynecologic care from the consensus project on chronic GvHD, starting at the age of 8 years, pubertal status, growth and serum levels of luteinizing hormone, follicle-stimulating hormone and estradiol should be evaluated every 6 months in girls. Estrogen therapy for the induction of puberty and the maximized growth and development of secondary sex characteristics should begin no later than the age of 15 years and not before 12 years. After the end of puberty, a short discontinuation of hormonal therapy is

| Table 1. | Requirements and expected success rates of fertility preservation strategies in pre- and post-pubertal girls/women and boys/men | | | | |
|---|---|---|---|---|---|
| Fertility preservation technique | Suggested timing before gonadotoxic therapy/conditioning | Requirements | Live birth rate[a,b] | Comments | Status of the therapy [c] |
| **Females**[d] | | | | | |
| Oocyte cryopreservation | 2 Weeks/3 weeks | Hormonal stimulation, vaginal ultrasound, transvaginal aspiration | ≈30–40%[37–39,84,88,89] | In postpubertal girls only In all infertility centers | Established |
| Ovarian tissue cryopreservation and tissue reimplantation | Half week/2 weeks | Laparoscopy general anesthesia | ≈30%[48,57,59] (insufficient data in prepubertal girls and no data/no reported offspring in blood-borne cancer) | In pre- and post-pubertal girls Tissue cannot be reimplanted in blood-borne cancer such as leukemia No proven techniques to use the tissue without reimplantation to date Only in highly specialized centers | In prepubertal girls: experimental, not proven In postpubertal girls: innovative In blood-borne cancer such as leukemia at any age: experimental |
| Combination of oocyte and ovarian tissue cryopreservation | 2.5 Weeks/3 weeks | See oocyte and ovarian tissue cryopreservation | ≈50%[47,48] | Only in postpubertal girls. Only in highly specialized centers | See oocyte and ovarian tissue cryopreservation |
| **Males**[e] | | | | | |
| Sperm cryopreservation | 1 Day/1 day | Masturbation ejaculation | >50%[27] | Only in postpubertal boys In all infertility centers | Established |
| Testicular biopsy for sperm cryopreservation (testicular sperm extraction (TESE)) | Half week/2 weeks | Local or spinal anesthesia | >50%[27] | In peri- and post-pubertal boys if masturbation is not possible In most infertility centers | Established |
| Testicular biopsy for stem cell cryopreservation | Half week/2 weeks | Local or spinal anesthesia | No data/no offspring reported[27] | For prepubertal boys (postpubertal boys after gonadotoxic therapy) Only in highly specialized centers | Experimental, not proven |

Reference numbers are reported as superscripts. [a]Following usage of all cryopreserved gametes and tissue. [b]Expected success rates are mainly extrapolated from adult patients affected with solid tumors; few data are available regarding patients undergoing hematopoietic cell transplantation during childhood or adolescence. [c]According to the criteria described by Provoost et al.[82] [d]Estimates are provided for usage in women with maximum 40 years of age after collection performed at < 35 years of age. [e]No age limits for male patients.

recommended to monitor whether ovarian function has recovered.[72]

A further aspect of post transplantation monitoring is the possible influence of chronic GvHD. In fact, pregnancies have been reported despite the presence of chronic GvHD, indicating that chronic GvHD and its therapy does not always impair fertility. Nonetheless, data on spontaneous and assisted conception are scarce.[73,74]

AMH levels, which are independent of the phase of the menstrual cycle, are currently used as a marker of ovarian reserve.[5,8] AMH levels may be considered in conjunction with follicle-stimulating hormone and estradiol for the identification of premature ovarian insufficiency among females older than 25 years.[75]

In pre- and peri-pubertal girls, no valid laboratory parameters exist to evaluate ovarian reserve. AMH levels are difficult to assess in the pediatric population because of missing reference values. Increasing levels of AMH, produced by granulosa cells in preantral and antral ovarian follicles, are observed in healthy girls from birth until puberty.[76–79] A baseline AMH level should also be measured after chemo/radiotherapy in girls who have survived cancer, even though the correlation between serum AMH and the fertility potential of the primordial follicles cohort has not been demonstrated in pre-/peri-pubertal girls.[49,80]

The detection of gonadal function in transplanted women, mostly after a reduced-intensity conditioning regimen, does not imply that menopause will not be accelerated thereafter because of the premature exhaustion of the finite number of germ cells present in the ovary. The detection of any residual ovarian function a few years after transplantation might suggest a benefit from ovarian or germ cell collection. Very young women may

think that they do not wish to become pregnant but should be aware that they may develop permanent sterility later.

## EXPECTED SUCCESS RATE ACCORDING TO SPECIFIC TECHNIQUES

The allocation of limited resources makes it important to understand the likelihood of success for each technique (Table 1). Moreover, informing patients about the expected success rates of each available fertility preservation option is a key point of fertility counseling.

Most data are extrapolated from adult patients with solid tumors. Estimates can be assessed with different methods and are subject to change in a rapidly evolving field. The recognition of what is 'established', 'innovative' or 'experimental' and 'not proven' to date, according to the definitions proposed by Provoost et al.,[81,82] is crucial in this setting. Outcome measures are expressed as the live birth rate for the use of all available samples, that is, after multiple attempts.

### Males

Stored semen can be used for in vitro fertilization, including intracytoplasmic injection or intrauterine insemination. The live birth rate would depend on semen quality, with intrauterine insemination being successful in up to 50% of cases and significantly correlated with the age and fertility of the female partner.[83,84]

Testicular biopsies have resulted in no pregnancies in humans because human spermatogonial cells cannot be matured to become spermatozoa to date.[32,85]

### Females

Stored mature oocytes are reported to yield at least one pregnancy out of eight in vitro fertilization attempts and up to 40% after multiple attempts for each patient. The success of the procedure depends on the number and the quality of the samples available that is variable for each patient.[34,37,38,83,84,86]

Reimplantation of postpubertal ovarian tissue has led to almost 100 babies to date.[56–59]

The combination of the two techniques, that is, collection of ovarian tissue and oocytes, may increase the pregnancy rate up to 50%.[47,48]

At present, postpubertal ovarian tissue without reimplantation cannot achieve in vitro maturation after thawing to obtain mature oocytes to fertilize.

Prepubertal ovaries have led to no pregnancies to date after reimplantation or in vitro maturation, which cannot be achieved in humans yet. One pregnancy has occurred by means of pubertal but premenarchal ovarian tissue autograft and natural fertilization.[61]

All fertility preservation approaches in pediatric transplantation, other than semen cryopreservation, are to be considered innovative or experimental, as defined above. It is important that cryopreserved gametes or gonadal tissues are analyzed and all findings are reported to facilitate research and allow progress in this developing field.

Considerably higher and earlier referral rates, specifically for chemo-naive patients, follow specific designated programs and optimize fertility preservation.

Financial issues have been investigated by means of a survey circulated among EBMT centers, as recently reported; as expected, policies widely vary throughout countries, with the costs of fertility preservation procedures being covered by public health systems, the government or charities in 55% of the cases, by health insurance companies in 42% and by families in 39%.[87]

## CONCLUSIONS

These consensus guidelines for fertility preservation are available for use by transplantation centers and health authorities for regulatory planning to support and finance fertility preservation projects.

Increasing awareness of fertility preservation techniques among hematologists, oncologists and transplanters will allow a progressively higher number of patients to benefit.

Moreover, as nurses spend more time at patients' bedsides than physicians and are often the first ones who are asked about infertility issues, nurse education about this topic is also important.

In summary, the fertility preservation procedures described above include:

- semen collection, which should be routinely performed in postpubertal boys at the diagnosis of any disease requiring therapies that can potentially impair fertility;
- testicular tissue collection, which is still experimental and has been unsuccessful to date;
- oocyte stimulation and transvaginal collection, which could be offered to postpubertal girls before facing gonadotoxic therapies;
- ovarian tissue collection, which may be offered when eligibility for transplantation (or other therapies with a high risk of infertility) is established to (1) prepubertal females, although its use is still experimental and has not been proven successful to date; (2) chemotherapy-naive postpubertal females, in whom fertility restoration can be successfully achieved after reimplantation; (3) postpubertal females with malignancy after exposure to chemotherapy who would fail to achieve oocyte mobilization, even though reimplantation is highly contraindicated in leukemia and ovarian tissue in vitro maturation and fertilization have not been successful to date.

Pregnancies after postpubertal ovarian tissue reimplantation have been reported; no pregnancy after reimplantation of prepubertal ovarian tissue or after in vitro maturation of pre- or post-pubertal ovarian tissue has been proven to date.

Possible future advances in reproductive medicine might change this scenario.

The infertility risks associated with novel conditioning regimens remains to be assessed. Rapid advances in reproductive medicine highlight the need for continuous updating. Any research assessing reproductive health will contribute to improved clinical care and increased long-term quality of life for transplant recipients. Fertility preservation projects should be prioritized in pediatric transplant settings.[21]

## CONFLICT OF INTEREST
The authors declare no conflict of interest.

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