Fertility preservation in children and adolescents

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Abstract

The quality of life of childhood cancer survivors has become a major concern as disease-free survival rates now reach greater than 80%. Moreover, the incidence of cancer diagnosis in children continues to increase in the large majority of countries. Oncological treatments, such as conditioning regimens before stem cell transplantation, can also be used for non-oncological indications such as haematological diseases (sickle cell anaemia, thalassemia) with similar long-term consequences on ovarian function. All guidelines now recommend that patients and their parents be informed about the risks of future infertility and premature ovarian insufficiency and that children at high risk be referred for fertility preservation. However, fertility preservation procedures remain experimental in children and should only be offered after careful evaluation of the risk/benefit balance by a multidisciplinary team. The only established procedure is oocyte or embryo cryopreservation but this cannot be applied in prepubertal patients and has limitations in adolescents. The most frequently used procedure is the cryopreservation of ovarian tissue that can be combined with ex vivo immature oocytes collection when feasible. Although this procedure has been performed in thousands of children worldwide, limited data are available on outcomes after transplantation of cryopreserved ovarian tissue in this population. Finally, more specific techniques have been addressed in this review such as ovarian transposition.

Key words: ovarian tissue cryopreservation, ovarian tissue transplantation, oocyte cryopreservation, fertility preservation, ovarian transposition

Definitions

Ovarian reserve: Number of quiescent follicles (primordial and primary) in the ovary. Ovarian reserve progressively decreases during life until menopause occurs. Ovarian reserve can be evaluated using pelvic ultrasound (Antral Follicular Count- AFC) and Hormonal levels (Anti-Mullerian Hormone- AMH, basal Follicle-stimulating Hormone- FSH and estradiol levels measured at the beginning of a natural menstrual cycle).

Premature ovarian insufficiency (POI): POI is a consequence of ovarian reserve depletion. POI is confirmed after at least 4 months of amenorrhea and two FSH values above 25IU/L (at 1 month interval) based on the ESHRE criteria.

Fertility preservation : procedures aiming to preserve fertility in patients who need treatments or surgery that can damage the ovary or who have been diagnosed with diseases that can induce premature ovarian insufficiency and infertility. Fertility preservation includes several strategies such as the reduction of the toxicity (ovarian transposition, radiation shield, pharmacoprotection) or cryopreservation of gametes (ovarian tissue, oocytes, sperm, testicular tissue) or embryos.

Take home messages

1. All children treated with gonadotoxic treatment, ovarian surgery or diagnosed with diseases that may accelerate follicular depletion and their parents should be inform about the risk of premature ovarian insufficiency. Consequences on puberty, menstruation, future contraception and fertility should be discussed.
2. Fertility preservation strategies should be offered in selected patients with a high risk of premature of ovarian insufficiency. Close collaboration with fertility specialist should be established to refer them within a short delay.
3. Oocytes cryopreservation is the first established option for fertility preservation in adults. However, efficacy, success rate and tolerance of the procedure need to be further investigated in young post-pubertal adolescent.
4. Cryopreservation of ovarian tissue is considered as experimental but it is the most frequently offered option in young patients. It is the only available option in prepubertal patients, or when oocytes cryopreservation is not feasible due to contraindication, timing or psychological issues.
5. Knowledge on fertility outcomes after ovarian tissue transplantation using ovarian tissue cryopreserved during chilhood very limited.

Clinical case and Practical clinical tips

A 15-years old girl was diagnosed with Hodgkin Lymphoma and was treated with 2 ABVD cycles (doxorubicin, bleomycin, vinblastine, and dacarbazine). The disease rapidly progressed despite the chemotherapy and oncologists decided to change treatment by using bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone regimen (BEACOPP escalated). She was referred with her parents for fertility preservation counseling**.**

- Inform them that fertility preservation was not recommended for low gonadotoxic treatment such as ABVD. However, BEACOPP escaladed including high dose of alkylating agents is an indication for fertility preservation.
- Offer them ovarian tissue cryopreservation option

Practical clinical tips

1. Ovarian tissue cryopreservation is the only option that can be offered when chemotherapy already started or after a low gonadotoxic chemotherapy regimen in children.

Young patient 18 years old diagnosed with AML, severe pancytopenia, required first line chemotherapy followed by HSCT.

- Inform the patients about the high risk of premature ovarian insufficiency after HSCT
- Discuss the fertility preservation options
- Propose ovarian tissue cryopreservation after the first line chemotherapy

Practical clinical tips

1. Oocyte cryopreservation is not feasible when the oncological treatment should start within a short delay or when health conditions contraindicated ovarian stimulation
2. Ovarian tissue cryopreservation should be proposed after the first line chemotherapy in leukemia patient in order to reduce the risk of disease contamination in the cryopreserved ovarian tissue**.**

Key readings

1. Corkum et al, 2019
2. Diesch et al, 2017
3. El Issaoui et al 2016
4. Demeestere et al, 2016
5. Irtan et al, 2013
6. Sklar et al, 2006

1. Introduction

Although a deceleration of the increase in the incidence of cancer in children has been observed, it is still increasing by 0.54% (0.44–0.65) per year in children (age 0–14 years) and the increase is quite clear for leukaemia, lymphoma, and malignant central nervous system (CNS) tumours that represent 70% of the cancer diagnosed in this population (Steliarova-Foucher *et al.,* 2018). Overall, 1 in 300 newborns will develop cancer before the age of 20. Due to the progress in oncological therapy, 80% of these patients are disease-free 5 years after diagnosis but two-thirds will face late side effects that can dramatically affect their long-term quality of life. In 2015, an International Society of Paediatric Oncology (SIOPE) Strategic Plan was presented at the European Society of Medical Oncology (ESMO) annual conference with the aim of improving both survival and quality of life in children diagnosed with cancer (SIOPE strategic plan : https://www.siope.eu/SIOPE_StrategicPlan2015/). The plan estimated that nearly half a million European citizens will be survivors of childhood cancer by 2020.

One of the major concerns of childhood cancer survivors is whether they will retain the future possibility of conception with their own gametes. The Childhood Cancer Survivor Study (CCSS), that included more than 3500 female cancer survivors diagnosed before the age of 21, confirmed the increased risk of infertility in this population (RR 1.48, 95% CI 1.23–1.78 compared to siblings) (Barton *et al.,* 2013). Factors associated with a high risk of infertility are pelvic irradiation and alkylating agent-based chemotherapy in both sexes (Brougham and Wallace 2005).

The future fertility of children affected by benign disease may also represent a major concern as some of these require similar high risk therapies such as conditioning regimens for haemopoietic stem cell transplantation (HSCT) in children with haematological benign diseases (e.g. sickle cell anaemia, thalassemia, granulomatosis). Finally, progress in genetics has provided early diagnosis for genetic disorders at risk of premature ovarian insufficiency such as Turner syndrome, blepharophimosis-ptosis-epicanthus syndrome (BPES), and galactosemia.

These young patients at risk of premature ovarian insufficiency due to a disease condition or gonadotoxic treatments for malignant or benign diseases and their parents should be informed about this risk and the possibility of preserving fertility (Coccia *et al.,* 2014, Gan and Spoudeas 2014).

2. Infertility risk in childhood cancer survivors

As female germ cells are not able to replicate after birth, the reproductive lifespan is determined by the number of primordial follicles (representing the ovarian reserve) at birth and the rate of follicular depletion through either atresia or ovulation. Ovarian failure occurs when the follicular pool is completely depleted (<1000 follicles) either naturally at menopause or earlier if the follicular reserve is prematurely lost through an accelerated atresia process (e.g. genetic disorders), exposure to gonadotoxic agents (e.g. chemotherapy and radiotherapy) or ovarian surgery. The risk can be evaluated according to the dose and nature of the gonadotoxic agents. The most gonadotoxic agents are alkylating drugs. Based on these criteria, chemotherapy is considered to be highly gonadotoxic when the alkylating agents score (AA score) reaches 3 or when the cyclophosphamide equivalent dose (CED) reaches 7 gr/m^2 or more (Table 1).

Table 1: Evaluation of the risk of premature ovarian insufficiency in children according to the dose and type of alkylating agents (Green et al, 2009).

Tools	Definition	Scale
Alkylating agents score (AA score)	Total dose per square meter of body surface area – Score are summed over all agents	0 no exposure 1 lower tertile dose 2 middle tertile dose 3 upper tertile dose
Cyclophosphamide equivalent dose (CED)	cumulative cyclophosphamide dose (mg/m^2)+0.244 (cumulative ifosfamide dose (mg/m^2)+ 0.857 (cumulative procarbazine dose (mg/m^2) + 14.286 (cumulative chlorambucil dose (mg/m^2)+15.0 (cumulative BCNU dose (mg/m^2)+16.0 (cumulative CCNU dose (mg/m^2)+40 (cumulative melphalan dose (mg/m^2)+50 (cumulative Thio-TEPA dose (mg/m^2) + 100 (cumulative nitrogen mustard dose (mg/m^2)+ 8.823 (cumulative busulfan dose (mg/m^2)	mg/m^2

BCNU, carmustine; CCNU, lomustine; TEPA, N,N′,N″-triethylenephosphoramide

In a long-term follow-up study of the Childhood Cancer Survivor Study cohort (CCSS) including almost 3000 patients, the authors identified different independent risk factors for non-surgical premature ovarian failure including: exposure to procarbazine ≥ 4,000mg/m^2 (OR 8.96; 95% CI 5.02–16.00), ovarian radiation (dose ≥500 cGy, OR 8.02; 95% CI 2.81–22.85), and HSCT (OR 6.35; 95% CI 1.19–33.93)(Levine *et al.,* 2018). In order to reduce mortality and morbidity, including long-term effects, associated with HSCT, alternative reduced-intensity conditioning (RIC) regimens have been implemented in non-malignant diseases based on combinations of treosulfan, fludarabine, and thymoglobulin, or alemtuzumab, fludarabine, and melphalan (Madden *et al.,* 2016). Limited data are available regarding long-term follow-up on ovarian function after RIC. In one study that included follow-up beyond 2 years of a cohort of 43

children treated with RIC, one out of 9 eligible patients for pubertal assessment faced premature ovarian failure (Madden *et al.,* 2016).

Acute ovarian failure is defined as permanent primary or secondary amenorrhea after a high-risk treatment. Based on follow-up of the CCSS cohort, it has been reported that 6.3% of female cancer survivors develop acute ovarian failure, defined as the loss of ovarian function within 5 years following diagnosis (Chemaitilly *et al.,* 2006). When oncological treatment was administered before puberty, children diagnosed with primary amenorrhea may require pubertal induction using hormonal treatment. The major causes of non-surgical acute ovarian failure include high dose alkylating agents for lymphoma or sarcoma, conditioning regimens before HSCT, and exposure to pelvic or spinal irradiation (Wallace *et al.,* 2004). Acute premature ovarian failure was observed in the majority of haematological patients treated with HSCT, especially when the conditioning regimen included busulfan or total body irradiation (Table 2). Gonadal failure is also common in cancer survivors treated with high dose chemotherapy and autologous stem cell rescue for other diseases such as neuroblastoma (Utriainen *et al.,* 2019).

Table 2: Ovarian function recovery rate after hematopoietic stem cell transplantation in young patients

Authors	Treatment	n	Age (range)	Ovarian recovery (%)	Follow-up
(Sanders et al., 1996)	Cy Bu/Cy Cy +TBI	103 73 532	28 y (13-58) 38 y (14-57) 28 y (11-58)	56 (54.3) 1 (1.3) 53 (10)	12-204 months (median 36)
(Sarafoglou et al., 1997)	Cy + TBI	16	Prepubertal	9 (56)	
(Teinturier et al., 1998)	CT CT (Bu)	11 10	5.8y (2-14.8) 12.7y (4.7-17.3)	7 (73) 0 (0)	14-156 months (median 84)
(Thibaud et al., 1998)	CT CT + TBI	8 23	10.3 y (3.2-17.5)	3 (37.5) 3 (13)	14-138 months (median 72)
(Bath et al., 1999)	CT + TBI	8	11.5y (5.9-15)	2 (25)	
(Couto-Silva et al., 2001)	CT+ TBI CT	22 5	7.3 y (1.5-13) 5.3 y (0.6-12.9)	3 (13.6) 2 (40)	
(Tauchmanova et al., 2002)	Bu/Cy	21	13-45 y	2 (5)	12-62 months (median 38)
(Jadoul et al., 2010)	CT+TBI CT	18 6	9.8±5.2 years (range 1.2–19.0)	4 (22) 6 (100)	15.5 ±5.5 years (range 3.3–33.7 years)

	CT (Bu)	11		5 (45)	

Cy=cyclophosphamide; Bu= Busulfan; TBI= Total Body Irradiation; CT= chemotherapy

When the gonadotoxicity of the therapy and/or the total dose is moderate, ovarian insufficiency may occur later during reproductive life. While most children will experience spontaneous puberty or will recover menstruation after chemotherapy, the ovarian reserve is often impaired by these moderate gonadotoxic treatments. Reduction of the ovarian reserve can induce premature ovarian failure (defined as cessation of menstruation and menopausal FSH levels before 40 years old), infertility, and low response to ovarian stimulation several years or decades after diagnosis. Sklar and al. have shown that childhood cancer survivors with spontaneous menstruation more than 5 years after diagnosis have a 13-fold increased risk of developing premature ovarian failure compared to siblings (Sklar *et al.,* 2006). Markers of the ovarian reserve in post-pubertal women include the measurement of anti-Mullerian hormone (AMH), follicle-stimulating hormone (FSH), and oestradiol (E2) blood levels, as well as follicular counting using ultrasound (antral follicular counting-AFC). Very limited data are available on the impact of treatment on the ovarian reserve in children. While physiological FSH levels remain low until puberty, AMH levels gradually increase to reach a peak at 24.5 years old (Kelsey *et al.,* 2011). Data on the impact of oncological treatment on the ovarian reserve remain limited. One analysis performed in a small cohort of children diagnosed with cancer (n=22) observed a correlation between AMH levels after 6-12 months and the gonadotoxicity of the chemotherapy regimen. This correlation was not observed with FSH or inhibin B as markers (Brougham *et al.,* 2012). The recovery rate was also associated with pre-treatment AMH levels in young patients (2.6% versus 11.9% per month for basal AMH levels <2 ng/ml or ≥2 ng/ml, respectively) (Dillon *et al.,* 2013). Compared to a control population, AMH levels were, thus, significantly lower in childhood cancer survivors (n=10, aged 16 to 34y) who spontaneously recovered menstruation while basal FSH levels were higher (n=11) (Bath *et al.,* 2002). Low AMH has been reported in around 30% of cancer survivors with normal menstruation and these women were still able to achieve pregnancy (Lie Fong *et al.,* 2010, Dillon *et al.,* 2013). A decline in AMH has also been correlated with increased CED score (cyclophosphamide >7.5 g/m^2), older age at exposure, and pelvic irradiation.

3. Fertility preservation

Access to appropriate counselling and fertility preservation procedures remains an important issue in children. In a recent European survey that included 38 centres that developed expertise in HSCT in children and adolescents, the authors reported that only 21 (55%) had a standardized program for fertility preservation procedures. A total of 39% and 16% of patients treated with HSCT received counselling and had a fertility preservation procedure performed,

respectively (Diesch *et al.,* 2017). However, fertility-related issues are a major concern for this young population and their parents (Korte *et al.,* 2019).

Fertility preservation counselling remains complex in children and adolescents. In prepubertal girls, the only option available is the cryopreservation of ovarian tissue that is still considered to be experimental. Fertility preservation strategies will be discussed according to pubertal status and disease.

3.1 Oocyte cryopreservation

The established option for fertility preservation in adults is the cryopreservation of mature oocytes or embryos after ovarian stimulation with gonadotropins and transvaginal oocyte collection. Ovarian stimulation (OS) takes around 10 days but adapted protocols have been implemented that start the treatment irrespective of cycle phase using gonadotropin releasing hormone (GnRH) antagonists to avoid spontaneous luteinizing hormone (LH) peaks. After around 10 days of OS, regular hormone level assessments, and control of follicular growth by transvaginal ultrasound (or transabdominal, if transvaginal evaluation is not feasible), ovulation can be triggered, either by GnRH agonist or by human chorionic gonadotropin (hCG), in order to collect mature oocytes 36 hours later. This protocol is considered standard in adults if there are no contraindications such as severe haematological disorders or high risk of thrombosis. Moreover, the delay should not compromise the efficacy of the oncological subsequent treatment. This option has also been proposed prior to bone marrow transplantation in teenage girls. In a case series of 8 patients aged between 14 and 18 years diagnosed with sickle cell anaemia, the authors reported mature oocyte recovery rates ranging from 1 to 30. Half of the patients had fewer than 8 mature oocytes cryopreserved, casting doubt upon the efficiency of using this procedure in adolescents (Lavery *et al.,* 2016). More recently, data for a cohort of 41 patients aged between 13 and 21 years who underwent ovarian stimulation for fertility preservation were reported (Manuel *et al.,* 2019). A total of 38 patients completed the cycle, with a median of 10 mature oocytes retrieved per patient (ranging from 0 to 25). Although the procedure is not feasible in prepubertal children, a case report of ovarian stimulation demonstrated the feasibility of the procedure in premenarchal patients who have already initiated puberty. The procedure was offered to a girl with myelodysplastic syndrome aged 13 years at diagnosis. Ovarian stimulation was initiated with 225 IU human menopausal gonadotrophin (hMG) using an GnRH antagonist after one week to avoid spontaneous LH surge. Follicular development was assessed by transabdominal ultrasound while oocyte collection was performed transvaginally under general anaesthesia after hCG triggering. Twenty oocytes were obtained of which only 8 were mature. The 12 immature oocytes were cultured for 20 hours, and 9 additional mature oocytes were cryopreserved after in vitro maturation (Reichman *et al.,* 2012). In a recent systematic review, the author identified 9 publications including oocyte cryopreservation in 20 young patients under 20 years of age,

illustrating the paucity of data in the field (Corkum *et al.,* 2019). There are several barriers limiting the use of this procedure in adolescents including access to appropriate fertility centres, the delay required for OS, the psychological impact of transvaginal ovarian assessment and oocyte collection, the risk of hyperstimulation, and the burden of daily subcutaneous injection during ovarian stimulation in this young population. All these limitations make the procedure complex in this population and often not feasible or not accepted. Moreover, data on outcomes and on the success rates of oocyte cryopreservation in the under 18 population are very limited. Successful live birth has been reported using cryopreserved oocytes at the age of 17 years (Kim and Hong 2011). Recent data have shown that the cumulative live birth rate of oncological adult patients who cryopreserved 5 and 8 oocytes before the age of 35 years reached 15.8% and 32%, respectively. Although the efficiency of the procedure is negatively correlated with age, the success rates of the procedure in premenarchal girls and adolescents are still unclear. Studies in sheep have shown that mature oocytes from prepubertal animals obtained after in vitro maturation (IVM) are less competent than oocytes from adults, mainly due to abnormal cytoplasmic maturation (Morton 2008, Leoni *et al.,* 2015). Incomplete nuclear maturation has been also described in prepubertal lambs, leading to low developmental competence and high rates of pregnancy arrest (Ptak *et al.,* 2006). The competence of oocytes collected before or shortly after menarche, as well as pregnancy outcomes using these oocytes, need to be further investigated in humans and the procedure should be proposed as an experimental approach to these young patients and their parents.

3.2 Ovarian tissue cryopreservation

Ovarian tissue cryopreservation (OTC) has been proposed as an alternative option to oocyte cryopreservation. It is the only available approach to cryopreservation of gametes in prepubertal girls and it is the most frequently offered option in the young post-pubertal population. Children and adolescents (<18 years old) represent around 25% of the patients who undergo the procedure in our centre. The major indications are benign or malignant haematological diseases such as sickle cell anaemia, or leukaemia or lymphoma requiring HSCT or high dose alkylating agents (Figure1).

OTC has the advantage of not requiring prior hormonal treatment or ovarian stimulation and of being performed at any time during the menstrual cycle. The delay between fertility preservation counselling and the procedure can be very short. Moreover, in contrast to oocyte cryopreservation, the procedure can be performed even if chemotherapy has already started. Recent data demonstrated that there were no differences in follicular density after first-line treatment in girls under the age of 18 years, although the ovarian surface area was reduced by 10% and 30% in young girls and adolescents, respectively (El Issaoui *et al.,* 2016). The long-term consequences of previous chemotherapy remain to be further explored but

pregnancies have been obtained after transplantation of ovarian tissue collected after first-line therapy (Demeestere *et al.,* 2007, Poirot *et al.,* 2019).

In OTC, the ovarian cortex is collected under general anaesthesia by laparoscopy or mini-laparotomy according to the age of the patient and the surgeon's skill level. The surgery can be performed in the paediatric centre and the tissue can be transported at 4°C for up to 24 hours before the cryopreservation procedure (Andersen *et al.,* 2018). In most countries, OTC procedures are centralized in centres with specific expertise and appropriate infrastructure. Therefore, a well-organized network with close collaboration between oncologists, surgeons, and fertility specialists is required to manage fertility preservation in this young population. A recent review reported partial oophorectomy in 43% of the procedures in young patients aged less than 20 years old, although unilateral oophorectomy is usually recommended in all prepubertal children who undergo OTC (Corkum *et al.,* 2019). Complications are rare but peri- and post-operative bleeding have been described (Jadoul *et al.,* 2010, Corkum *et al.,* 2019). The safety of anaesthesia in children has also been dramatically improved in the past few decades. Nevertheless, the OTC procedure should always be associated with another intervention such as central venous catheter insertion, biopsy or tumour resection, when feasible, in order to avoid repeated anaesthesia, especially in very young children (Jadoul *et al.,* 2010). Higher risk of respiratory failure has been observed during anaesthesia in children and risk factors must be identified during pre-anaesthetic assessment (von Ungern-Sternberg *et al.,* 2010). Chemotherapy treatment can be usually initiated the day after OTC.

The procedure aims to cryopreserve a large number of the non-growing follicles (primordial and primary follicular pool) that constitute the ovarian reserve. Follicular density is negatively correlated with age and is particularly high in children. A predictive model has been established to estimate follicular density and ovarian volume according to age. At 16 years old, the number of non-growing follicles and the ovarian volume were 147,912 and 6358 mm^3 respectively, while these values dropped to 98,106 and 7695 mm^3 at 21 years old, respectively (McLaughlin *et al.,* 2015). Poirot et al, (2002) evaluated follicular density in a cohort of young patients who underwent OTC. In patients under 7 years of age, the mean number of non-growing follicles was 20.36/mm^2 (n=6) and this decreased to 4.13/mm^2 for patients between 10 and 15 years old (n=8)(Poirot *et al.,* 2002). However, follicular density varied dramatically from one fragment to another. In two patients 12 years of age, ovarian biopsies revealed a follicular pool from 110 to 1138 with 50% to 93% of primordial follicles within the tissue fragment (Albamonte *et al.,* 2019).

The balance between the risks and benefits of the procedure should always been carefully evaluated before surgery, including the additional risk of ovarian insufficiency due to the procedure. A subgroup of 60 girls were evaluated for the risk of premature ovarian failure in a large Danish cohort of 176 patients aged between 12 and 18 years at OTC. When they reached

a mean of 21.1 years old, 43% and 10% received hormone replacement therapy and oral contraception, respectively. A total of 45% reported normal regular menstruation (Jensen *et al.,* 2017). For patients under 12 years of age at OTC, 71% required medical puberty induction. The impact of oophorectomy or ovarian biopsy on the future risk of premature ovarian insufficiency is still unclear. After excluding patients facing acute ovarian failure, childhood cancer survivors who underwent unilateral oophorectomy reached menopause 7 years earlier than those who did not (median:42 years, 95% CI:40-46 versus 49 years, 95% CI:48-50) (Thomas-Teinturier *et al.,* 2013). These women have a 3.7-fold increased risk of menopause. In patients who underwent fertility-sparing surgery for localized ovarian cancer, younger age at diagnosis was also associated with a higher risk of early menopause (Letourneau *et al.,* 2015). Moreover, unilateral oophorectomy at a young age is associated with an increased risk of surgery-associated menopause during reproductive life in the event of diseases affecting the remaining ovary (e.g. endometrioma, recurrent ovarian cyst, tumour, torsion). On the other hand, ovarian cortex biopsy is more complex to perform as coagulation should be avoided to prevent damage of the cryopreserved cortex as well as the remaining ovary. Bleeding on the remaining ovary should be managed with caution to maintain an optimal ovarian reserve. For prepubertal girls, this procedure is not feasible and unilateral oophorectomy is usually performed (Poirot *et al.,* 2019).

Slow freezing is the standard procedure to cryopreserve ovarian tissue in adults and children. After thawing and xenograft into mice, around 80% of the follicles survive (Newton *et al.,* 1996). Vitrification has become the standard for oocytes and embryos, and has recently emerged as a potential technique for cryopreservation of ovarian tissue. In a meta-analysis comparing both techniques, the authors concluded that the techniques were similar in terms of proportion of intact follicles but vitrification was associated with less DNA damage and better stromal cells morphology (Shi *et al.,* 2017). However, protocols were not standardized, making comparisons difficult and the number of live births obtained after transplantation remains limited.

3.2.1 Outcomes after transplantation of cryopreserved ovarian tissue

At present, orthotopic and heterotopic transplantations are the only available options for restoring fertility using cryopreserved ovarian tissue. More than 130 live births have been reported after transplantation of ovarian tissue collected in adults with an overall success rate of around 40% (Gellert *et al.,* 2018). Data regarding the outcomes of transplantation of cryopreserved ovarian tissue collected during childhood remain much more limited. A first transplantation was reported for pubertal induction in a prepubertal girl aged 10 years at the time of OTC. The procedure was performed before HSCT for sickle cell anaemia and the patient came back 27 months later to use the cryopreserved tissue for endocrine restoration. Another case of pubertal induction after transplantation of cryopreserved ovarian tissue was reported in a patient aged 9 years at OTC before she was treated for Ewing sarcoma (Ernst *et*

al., 2013). In both cases, hormonal function was temporally observed with puberty progression, although the indication has raised several concerns. First, the procedure is invasive and patients can be at risk of malignant cell transmission in cancer survivors whereas safe hormonal medication is available as an established method for induction of puberty and menarche. Moreover, the duration of restoration of ovarian function after transplantation is limited and ovarian tissue should be kept for later fertility restoration. Finally, sudden unphysiological rises in oestradiol may induce accelerated puberty, leading to premature growth arrest and overt weight gain (Andersen *et al.,* 2013, von Wolff *et al.,* 2016). Therefore, autologous transplantation of ovarian tissue is not recommended for this purpose.

Only 3 cases of successful restoration of fertility after autologous transplantation of ovarian tissue cryopreserved before menarche have been reported. The first live birth was reported in 2015. Unilateral oophorectomy was performed before HSCT for sickle cell anaemia in a patient aged 13 years and 11 months. She had initiated puberty but not menarche. Menstruations were induced 18 months later by hormonal replacement therapy as she was diagnosed with acute premature ovarian failure. Ten years later, she underwent ovarian tissue transplantation to restore her fertility after confirmation of her menopausal status. Spontaneous menstruations occurred after 5 months and she delivered two healthy babies after natural conceptions 2 and 5 years following the grafting procedure (Demeestere *et al.,* 2015). A second live birth after ovarian tissue transplantation was reported in a woman aged 10 years at OTC (Matthews *et al.,* 2018). The pregnancy was obtained after an intracytoplasmic sperm injection (ICSI) procedure. Three other patients who underwent ovarian tissue transplantation using tissue cryopreserved during childhood have been reported on in the literature but no pregnancies were obtained: one faced disease recurrence 4 months after the transplantation procedure, one did not have restoration of endocrine function after a first graft, and one was still being monitored at the time of the report (Corkum *et al.,* 2019, Poirot *et al.,* 2019). In the post-pubertal population under 21 years at OTC, a total of 15 ovarian tissue transplantations have been reported. Overall, 56% of the patients have had at least one live birth and 60% of these were naturally conceived (Corkum *et al.,* 2019).

Several issues have to be considered regarding the ovarian tissue transplantation procedure. First, the risk of recurrence of the disease due to potential transmission of the malignant cells must be carefully assessed. This risk depends on the type and the localization of the disease and is considered to be high in leukaemia, neuroblastoma, Burkitt lymphoma, and in neoplasias with distant metastases or involving the pelvis (Dolmans *et al.,* 2013). Leukaemia represents one of the major indications for OTC in children. The presence of neoplastic cells within the ovarian tissue was observed in more than half of the cases after molecular detection (Dolmans *et al.,* 2013). However, this risk can be reduced when the ovarian tissue is

cryopreserved after first-line chemotherapy (Greve *et al.,* 2012). A first successful transplantation of ovarian tissue collected at the time of complete remission after first-line chemotherapy was recently reported (Shapira *et al.,* 2018). Nevertheless, caution should be taken in patients at high risk and appropriate analysis of the ovarian cortex and residual medulla should be performed using the most sensitive techniques, such as PCR, when available. Second, outcomes after transplantation of ovarian tissue from prepubertal children remain uncertain. Although the tissue contains a large number of non-growing follicles, high rates of abnormal follicles have been described in young children. After analysis of ovarian cortex from 25 patients aged from 4 to 39 years, Westergaard and colleagues (2007) reported an increase in the diameter of the oocytes with age as well as in the number of granulosa cells surrounding primary follicles while oocyte nuclear diameter seems to be unaffected by age (Westergaard *et al.,* 2007). Another study observed a higher rate of abnormalities in the primordial follicles from prepubertal compared to pubertal girls including the absence of a nucleus, poor vesicle germinal definition, or multinucleated follicles (abnormality rates of 19.4±5.6% of oocytes within non-growing follicles in tissue from the pre-pubertal group and 4.8±1.6% of non-growing follicles from the pubertal group). After in vitro culture, the follicles from the prepubertal group also exhibited limited growth activation and did not grow at the same rate as follicles from the pubertal group (Anderson *et al.,* 2014). Finally, animal studies have demonstrated that oocytes from prepubertal growing follicles are less competent, resulting in a lower blastocyst development rate (Table 3). This may be explained by differences in the distribution and density of mitochondria (Leoni *et al.,* 2015).

Table 3: Characteristics of follicles from prepubertal ovaries observed in human or animal studies

CHARACTERISTICS	**REF**
Presence of high density of follicles at all stages (except pre-ovulatory follicles)	(Peters *et al.,* 1978)
Diameter of primordial/transitory follicles/oocytes increases with woman's age (from age 4 through mid-30's) but nucleus size is unaffected by age	(Westergaard *et al.,* 2007)
Number of granulosa cells in small follicles increases with age	(Westergaard *et al.,* 2007)
High rate of abnormal follicles	(Anderson *et al.,* 2014) (Luyckx *et al.,* 2013)

Growing follicles less sensitive to FSH	(Yang *et al.,* 2009)
Growing follicles/oocytes obtained after in vitro culture are smaller than those from young adults	(Xu *et al.,* 2010, Anderson *et al.,* 2014)
Delay in oocyte maturation Low ATP oocyte concentrations (fewer mitochondria in mature oocytes) Higher spontaneous parthenogenetic activation rate Higher polyspermia rate	(Kochhar *et al.,* 2002) (Palmerini *et al.,* 2014) (Leoni *et al.,* 2015)
Lower blastocyst development but similar morphology of the embryo Reduction in global methylation High pregnancy loss	Leoni et al. 2006 Ptak et al. , 2006

3.2.2 Combined ovarian tissue and oocyte cryopreservation procedure

Ovarian tissue cryopreservation can be combined with ex-vivo immature oocyte collection as antral follicles are observed even at prepubertal ages (Abir *et al.,* 2016, Fasano *et al.,* 2017). These oocytes can be in vitro matured and cryopreserved for future in vitro fertilization. Although few pregnancies have been obtained using in vitro maturation of immature oocytes collected ex vivo in adult patients, the success rate is probably very low. A recent report of outcomes after thawing 35 embryos and 8 mature oocytes from immature oocytes collected during ovarian tissue cryopreservation procedures in adults showed a survival rate of 82% for the thawed embryos but only one pregnancy was obtained (Kedem *et al.,* 2018). The success rate may be even lower in children as lower maturation rates have been observed compared to immature oocytes from adults (10.3% versus 28.8%). Moreover, the maturation process was delayed as the majority of the oocytes matured after 48h and 24h in prepubertal versus post-pubertal patients, respectively (Fasano *et al.,* 2017). Animal studies have also demonstrated low and delayed maturation rates when immature oocytes were collected in prepubertal animals (Table 3). Finally, heathy oocytes are usually not found in children aged under 5 years (Fasano *et al.,* 2017).

3.3 Ovarian transposition

Ovarian transposition is the first procedure to become available for situations when high dose pelvic irradiation is required in children treated for malignancies such as pelvic sarcoma or Hodgkin lymphoma. However, only 6 studies have been reported in this population (Corkum

et al., 2019). It is usually performed after adjuvant therapy concomitantly with tumour resection (Irtan *et al.,* 2013). The ovaries are relocalised at a distance from the irradiation field to avoid direct damage using laparotomy or laparoscopy. The success rate of the procedure in this population is unclear. A failure rate of 10%-14% was previously reported in children (Irtan *et al.,* 2013). However, long-term follow-up did not demonstrate a real benefit in terms of premature ovarian insufficiency in the St. Jude Lifetime Cohort Study (SJLIFE) treated for Hodgkin lymphoma, most probably due to the association of gonadotoxic chemotherapy (Fernandez-Pineda *et al.,* 2018). Another long-term study observed ovarian function in all 18 young patients who underwent ovarian transposition after a mean follow-up of 8.6±0.9 years and two pregnancies occurred, although 15 out of 18 patients received chemotherapy (Thibaud *et al.,* 1992).

Complications were observed including intestinal occlusion, dyspareunia, functional ovarian cysts, and pelvic adhesions with tubal obstruction.

3.4 Pharmacoprotective therapy

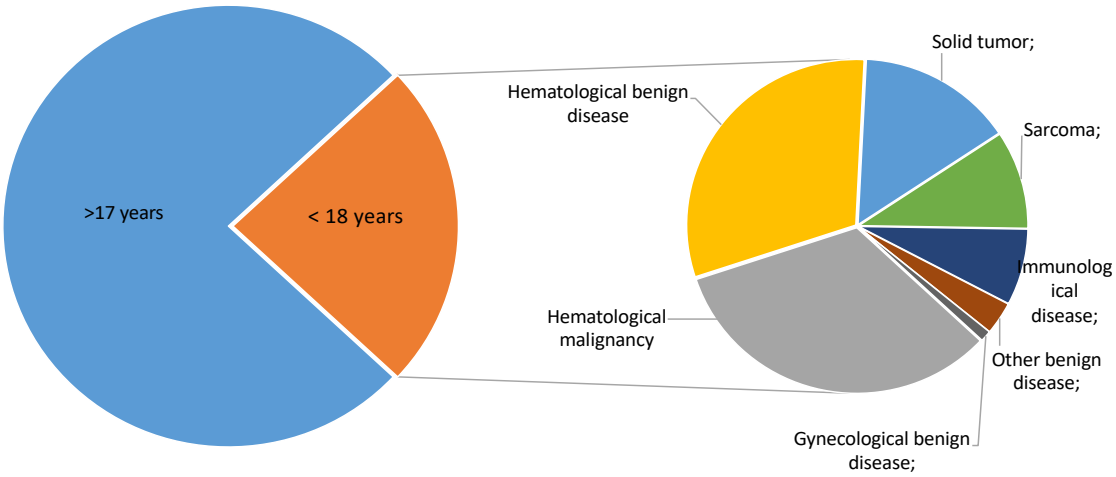
Pharmacoprotective therapy aims to reduce the gonadotoxic effects of chemotherapy. Although it appears to be a very attractive approach as it is non-invasive and allows spontaneous restoration of ovarian function after oncological treatment, only one drug has been tested in clinical trials for this indication. Gonadotropin-releasing hormone agonist (GnRHa) has been used in post-pubertal patients to mimic the prepubertal status and reduce follicular activation during chemotherapy. Others have suggested that it may act directly on the ovaries through GnRH receptors to reduce apoptosis. However, none of these potential mechanisms of action have been demonstrated by experimental studies (Lambertini *et al.,* 2019). Moreover, there are no rational reasons to offer this option in prepubertal children. All randomized controlled trials including adult patients have demonstrated controversial results. While results obtained in lymphoma patients did not confirm the efficacy of the treatment to prevent premature ovarian insufficiency, data in breast cancer showed a reduction in amenorrhea rates after short-term follow-up (Demeestere *et al.,* 2016, Lambertini *et al.,* 2018). Overall, the recent recommendations stated that this option could be offered in breast cancer patients but should not replace other fertility procedures.

4. Conclusion

Although counselling regarding the fertility issue in children is complex and options are limited, fertility preservation should be offered to all young patients undergoing highly gonadotoxic treatment. The only available option in prepubertal children is ovarian tissue cryopreservation. Oocyte cryopreservation can be considered in adolescents but the procedure may be more

complex to manage and better efficacy has not been demonstrated compared to ovarian tissue cryopreservation. Decisions should take into consideration the patient's age and maturity, the disease type and localisation, as well as the oncological treatments and imperatives.

Figure 1: Proportion and Indication of ovarian tissue cryopreservation in children at Erasme Hospital, Brussels, Belgium (1999-2019)



References

Abir R, Ben-Aharon I, Garor R, Yaniv I, Ash S, Stemmer SM, Ben-Haroush A, Freud E, Kravarusic D, Sapir O *et al.* Cryopreservation of in vitro matured oocytes in addition to ovarian tissue freezing for fertility preservation in paediatric female cancer patients before and after cancer therapy. *Hum Reprod* 2016;**31**:750-62.

Albamonte MI, Albamonte MS, Bou-Khair RM, Zuccardi L and Vitullo AD. The ovarian germinal reserve and apoptosis-related proteins in the infant and adolescent human ovary. *J Ovarian Res* 2019;**12**:22.

Andersen CY, Bollerup AC and Kristensen SG. Defining quality assurance and quality control measures in connection with ovarian tissue cryopreservation and transplantation: a call to action. *Hum Reprod* 2018;**33**:1201-1204.

Andersen CY, Ernst E, Kjaersgaard M, Birkebaek NH and Clausen N. Response to the letter to editor from Richard A. Anderson and co-workers. *Eur J Cancer* 2013;**49**:2962-3.

Anderson RA, McLaughlin M, Wallace WH, Albertini DF and Telfer EE. The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence. *Hum Reprod* 2014;**29**:97-106.

Barton SE, Najita JS, Ginsburg ES, Leisenring WM, Stovall M, Weathers RE, Sklar CA, Robison LL and Diller L. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. *Lancet Oncol* 2013;**14**:873-81.

Bath LE, Critchley HO, Chambers SE, Anderson RA, Kelnar CJ and Wallace WH. Ovarian and uterine characteristics after total body irradiation in childhood and adolescence: response to sex steroid replacement. *Br J Obstet Gynaecol* 1999;**106**:1265-72.

Bath LE, Wallace WH and Critchley HO. Late effects of the treatment of childhood cancer on the female reproductive system and the potential for fertility preservation. *Bjog* 2002;**109**:107-14.

Brougham MF, Crofton PM, Johnson EJ, Evans N, Anderson RA and Wallace WH. Anti-Mullerian hormone is a marker of gonadotoxicity in pre- and postpubertal girls treated for cancer: a prospective study. *J Clin Endocrinol Metab* 2012;**97**:2059-67.

Brougham MF and Wallace WH. Subfertility in children and young people treated for solid and haematological malignancies. *Br J Haematol* 2005;**131**:143-55.

Chemaitilly W, Mertens AC, Mitby P, Whitton J, Stovall M, Yasui Y, Robison LL and Sklar CA. Acute ovarian failure in the childhood cancer survivor study. *J Clin Endocrinol Metab* 2006;**91**:1723-8.

Coccia PF, Pappo AS, Altman J, Bhatia S, Borinstein SC, Flynn J, Frazier AL, George S, Goldsby R, Hayashi R *et al.* Adolescent and young adult oncology, version 2.2014. *J Natl Compr Canc Netw* 2014;**12**:21-32; quiz 32.

Corkum KS, Rhee DS, Wafford QE, Demeestere I, Dasgupta R, Baertschiger R, Malek MM, Aldrink JH, Heaton TE, Weil BR *et al.* Fertility and hormone preservation and restoration for female children and adolescents receiving gonadotoxic cancer treatments: A systematic review. *J Pediatr Surg* 2019;**54**:2200-2209.

Couto-Silva AC, Trivin C, Thibaud E, Esperou H, Michon J and Brauner R. Factors affecting gonadal function after bone marrow transplantation during childhood. *Bone Marrow Transplant* 2001;**28**:67-75.

Demeestere I, Brice P, Peccatori FA, Kentos A, Dupuis J, Zachee P, Casasnovas O, Van Den Neste E, Dechene J, De Maertelaer V *et al.* No Evidence for the Benefit of Gonadotropin-Releasing Hormone Agonist in Preserving Ovarian Function and Fertility in Lymphoma

Survivors Treated With Chemotherapy: Final Long-Term Report of a Prospective Randomized Trial. *J Clin Oncol* 2016;**34**:2568-74.

Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C, Delbaere A, Devreker F and Ferster A. Live birth after autograft of ovarian tissue cryopreserved during childhood. *Hum Reprod* 2015;**30**:2107-9.

Demeestere I, Simon P, Emiliani S, Delbaere A and Englert Y. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. *Oncologist* 2007;**12**:1437-42.

Diesch T, Rovo A, von der Weid N, Faraci M, Pillon M, Dalissier A, Dalle JH and Bader P. Fertility preservation practices in pediatric and adolescent cancer patients undergoing HSCT in Europe: a population-based survey. *Bone Marrow Transplant* 2017;**52**:1022-1028.

Dillon KE, Sammel MD, Ginsberg JP, Lechtenberg L, Prewitt M and Gracia CR. Pregnancy after cancer: results from a prospective cohort study of cancer survivors. *Pediatr Blood Cancer* 2013;**60**:2001-6.

Dillon KE, Sammel MD, Prewitt M, Ginsberg JP, Walker D, Mersereau JE, Gosiengfiao Y and Gracia CR. Pretreatment antimullerian hormone levels determine rate of posttherapy ovarian reserve recovery: acute changes in ovarian reserve during and after chemotherapy. *Fertil Steril* 2013;**99**:477-83.

Dolmans MM, Luyckx V, Donnez J, Andersen CY and Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril* 2013;**99**:1514-22.

El Issaoui M, Giorgione V, Mamsen LS, Rechnitzer C, Birkebaek N, Clausen N, Kelsey TW and Andersen CY. Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years. *Fertil Steril* 2016;**106**:1757-1762 e1.

Ernst E, Kjaersgaard M, Birkebaek NH, Clausen N and Andersen CY. Case report: stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. *Eur J Cancer* 2013;**49**:911-4.

Fasano G, Dechene J, Antonacci R, Biramane J, Vannin AS, Van Langendonckt A, Devreker F and Demeestere I. Outcomes of immature oocytes collected from ovarian tissue for cryopreservation in adult and prepubertal patients. *Reprod Biomed Online* 2017;**34**:575-582.

Fernandez-Pineda I, Davidoff AM, Lu L, Rao BN, Wilson CL, Srivastava DK, Klosky JL, Metzger ML, Krasin MJ, Ness KK *et al.* Impact of ovarian transposition before pelvic irradiation on

ovarian function among long-term survivors of childhood Hodgkin lymphoma: A report from the St. Jude Lifetime Cohort Study. *Pediatr Blood Cancer* 2018;**65**:e27232.

Gan HW and Spoudeas HA. Long-term follow-up of survivors of childhood cancer (SIGN Clinical Guideline 132). *Arch Dis Child Educ Pract Ed* 2014;**99**:138-43.

Gellert SE, Pors SE, Kristensen SG, Bay-Bjorn AM, Ernst E and Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. *J Assist Reprod Genet* 2018;

Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sorensen SD, Rosendahl M, Ralfkiaer E and Andersen CY. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. *Blood* 2012;**120**:4311-6.

Irtan S, Orbach D, Helfre S and Sarnacki S. Ovarian transposition in prepubescent and adolescent girls with cancer. *Lancet Oncol* 2013;**14**:e601-8.

Jadoul P, Dolmans MM and Donnez J. Fertility preservation in girls during childhood: is it feasible, efficient and safe and to whom should it be proposed? *Hum Reprod Update* 2010;

Jensen AK, Rechnitzer C, Macklon KT, Ifversen MR, Birkebaek N, Clausen N, Sorensen K, Fedder J, Ernst E and Andersen CY. Cryopreservation of ovarian tissue for fertility preservation in a large cohort of young girls: focus on pubertal development. *Hum Reprod* 2017;**32**:154-164.

Kedem A, Yerushalmi GM, Brengauz M, Raanani H, Orvieto R, Hourvitz A and Meirow D. Outcome of immature oocytes collection of 119 cancer patients during ovarian tissue harvesting for fertility preservation. *J Assist Reprod Genet* 2018;**35**:851-856.

Kelsey TW, Wright P, Nelson SM, Anderson RA and Wallace WH. A validated model of serum anti-mullerian hormone from conception to menopause. *PLoS One* 2011;**6**:e22024.

Kim TJ and Hong SW. Successful live birth from vitrified oocytes after 5 years of cryopreservation. *J Assist Reprod Genet* 2011;**28**:73-6.

Kochhar HP, Wu B, Morris LH, Buckrell BC, Pollard JW, Basrur PK and King WA. Maturation status, protein synthesis and developmental competence of oocytes derived from lambs and ewes. *Reprod Domest Anim* 2002;**37**:19-25.

Korte E, Schilling R, Balcerek M, Byrne J, Dirksen U, Herrmann G, Kepak T, Klco-Brosius S, Kruseova J, Kunstreich M *et al.* Fertility-Related Wishes and Concerns of Adolescent Cancer Patients and Their Parents. *J Adolesc Young Adult Oncol* 2019;

Lambertini M, Horicks F, Del Mastro L, Partridge AH and Demeestere I. Ovarian protection with gonadotropin-releasing hormone agonists during chemotherapy in cancer patients: From biological evidence to clinical application. *Cancer Treat Rev* 2019;**72**:65-77.

Lambertini M, Moore HCF, Leonard RCF, Loibl S, Munster P, Bruzzone M, Boni L, Unger JM, Anderson RA, Mehta K *et al.* Gonadotropin-Releasing Hormone Agonists During Chemotherapy for Preservation of Ovarian Function and Fertility in Premenopausal Patients With Early Breast Cancer: A Systematic Review and Meta-Analysis of Individual Patient-Level Data. *J Clin Oncol* 2018;**36**:1981-1990.

Lavery SA, Islam R, Hunt J, Carby A and Anderson RA. The medical and ethical challenges of fertility preservation in teenage girls: a case series of sickle cell anaemia patients prior to bone marrow transplant. *Hum Reprod* 2016;**31**:1501-7.

Leoni GG, Palmerini MG, Satta V, Succu S, Pasciu V, Zinellu A, Carru C, Macchiarelli G, Nottola SA, Naitana S *et al.* Differences in the Kinetic of the First Meiotic Division and in Active Mitochondrial Distribution between Prepubertal and Adult Oocytes Mirror Differences in their Developmental Competence in a Sheep Model. *PLoS One* 2015;**10**:e0124911.

Letourneau J, Chan J, Salem W, Chan SW, Shah M, Ebbel E, McCulloch C, Chen LM, Cedars M and Rosen M. Fertility Sparing Surgery for Localized Ovarian Cancers Maintains an Ability to Conceive, but is Associated With Diminished Reproductive Potential. *J Surg Oncol* 2015;**112**:26-30.

Levine JM, Whitton JA, Ginsberg JP, Green DM, Leisenring WM, Stovall M, Robison LL, Armstrong GT and Sklar CA. Nonsurgical premature menopause and reproductive implications in survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. *Cancer* 2018;**124**:1044-1052.

Lie Fong S, van den Heuvel-Eibrink MM, Eijkemans MJ, Schipper I, Hukkelhoven CW and Laven JS. Pregnancy outcome in female childhood cancer survivors. *Hum Reprod* 2010;**25**:1206-12.

Luyckx V, Scalercio S, Jadoul P, Amorim CA, Soares M, Donnez J and Dolmans MM. Evaluation of cryopreserved ovarian tissue from prepubertal patients after long-term xenografting and exogenous stimulation. *Fertil Steril* 2013;**100**:1350-7.

Madden LM, Hayashi RJ, Chan KW, Pulsipher MA, Douglas D, Hale GA, Chaudhury S, Haut P, Kasow KA, Gilman AL *et al.* Long-Term Follow-Up after Reduced-Intensity Conditioning and Stem Cell Transplantation for Childhood Nonmalignant Disorders. *Biol Blood Marrow Transplant* 2016;**22**:1467-1472.

Manuel SL, Moravek MB, Confino R, Smith KN, Lawson AK, Klock SC and Pavone ME. Ovarian stimulation is a safe and effective fertility preservation option in the adolescent and young adult population. *J Assist Reprod Genet* 2019;

Matthews SJ, Picton H, Ernst E and Andersen CY. Successful pregnancy in a woman previously suffering from beta-thalassemia following transplantation of ovarian tissue cryopreserved before puberty. *Minerva Ginecol* 2018;**70**:432-435.

McLaughlin M, Kelsey TW, Wallace WH, Anderson RA and Telfer EE. An externally validated age-related model of mean follicle density in the cortex of the human ovary. *J Assist Reprod Genet* 2015;**32**:1089-95.

Morton KM. Developmental capabilities of embryos produced in vitro from prepubertal lamb oocytes. *Reprod Domest Anim* 2008;**43 Suppl 2**:137-43.

Newton H, Aubard Y, Rutherford A, Sharma V and Gosden R. Low temperature storage and grafting of human ovarian tissue. *Hum Reprod* 1996;**11**:1487-91.

Palmerini MG, Nottola SA, Leoni GG, Succu S, Borshi X, Berlinguer F, Naitana S, Bekmukhambetov Y and Macchiarelli G. In vitro maturation is slowed in prepubertal lamb oocytes: ultrastructural evidences. *Reprod Biol Endocrinol* 2014;**12**:115.

Peters H, Byskov AG and Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab* 1978;**7**:469-85.

Poirot C, Brugieres L, Yakouben K, Prades-Borio M, Marzouk F, de Lambert G, Pacquement H, Bernaudin F, Neven B, Paye-Jaouen A *et al.* Ovarian tissue cryopreservation for fertility preservation in 418 girls and adolescents up to 15 years of age facing highly gonadotoxic treatment. Twenty years of experience at a single center. *Acta Obstet Gynecol Scand* 2019;**98**:630-637.

Poirot C, Fortin A, Lacorte JM, Akakpo JP, Genestie C, Vernant JP, Brice P, Morice P, Leblanc T, Gabarre J *et al.* Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. *Hum Reprod* 2019;**34**:1083-1094.

Poirot C, Vacher-Lavenu MC, Helardot P, Guibert J, Brugieres L and Jouannet P. Human ovarian tissue cryopreservation: indications and feasibility. *Hum Reprod* 2002;**17**:1447-52.

Ptak G, Matsukawa K, Palmieri C, Della Salda L, Scapolo PA and Loi P. Developmental and functional evidence of nuclear immaturity in prepubertal oocytes. *Hum Reprod* 2006;**21**:2228-37.

Reichman DE, Davis OK, Zaninovic N, Rosenwaks Z and Goldschlag DE. Fertility preservation using controlled ovarian hyperstimulation and oocyte cryopreservation in a premenarcheal female with myelodysplastic syndrome. *Fertil Steril* 2012;**98**:1225-8.

Sanders JE, Hawley J, Levy W, Gooley T, Buckner CD, Deeg HJ, Doney K, Storb R, Sullivan K, Witherspoon R *et al.* Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. *Blood* 1996;**87**:3045-52.

Sarafoglou K, Boulad F, Gillio A and Sklar C. Gonadal function after bone marrow transplantation for acute leukemia during childhood. *J Pediatr* 1997;**130**:210-6.

Shapira M, Raanani H, Barshack I, Amariglio N, Derech-Haim S, Marciano MN, Schiff E, Orvieto R and Meirow D. First delivery in a leukemia survivor after transplantation of cryopreserved ovarian tissue, evaluated for leukemia cells contamination. *Fertil Steril* 2018;**109**:48-53.

Shi Q, Xie Y, Wang Y and Li S. Vitrification versus slow freezing for human ovarian tissue cryopreservation: a systematic review and meta-anlaysis. *Sci Rep* 2017;**7**:8538.

Sklar CA, Mertens AC, Mitby P, Whitton J, Stovall M, Kasper C, Mulder J, Green D, Nicholson HS, Yasui Y *et al.* Premature menopause in survivors of childhood cancer: a report from the childhood cancer survivor study. *J Natl Cancer Inst* 2006;**98**:890-6.

Steliarova-Foucher E, Fidler MM, Colombet M, Lacour B, Kaatsch P, Pineros M, Soerjomataram I, Bray F, Coebergh JW, Peris-Bonet R *et al.* Changing geographical patterns and trends in cancer incidence in children and adolescents in Europe, 1991-2010 (Automated Childhood Cancer Information System): a population-based study. *Lancet Oncol* 2018;**19**:1159-1169.

Tauchmanova L, Selleri C, Rosa GD, Pagano L, Orio F, Lombardi G, Rotoli B and Colao A. High prevalence of endocrine dysfunction in long-term survivors after allogeneic bone marrow transplantation for hematologic diseases. *Cancer* 2002;**95**:1076-84.

Teinturier C, Hartmann O, Valteau-Couanet D, Benhamou E and Bougneres PF. Ovarian function after autologous bone marrow transplantation in childhood: high-dose busulfan is a major cause of ovarian failure. *Bone Marrow Transplant* 1998;**22**:989-94.

Thibaud E, Ramirez M, Brauner R, Flamant F, Zucker JM, Fekete C and Rappaport R. Preservation of ovarian function by ovarian transposition performed before pelvic irradiation during childhood. *J Pediatr* 1992;**121**:880-4.

Thibaud E, Rodriguez-Macias K, Trivin C, Esperou H, Michon J and Brauner R. Ovarian function after bone marrow transplantation during childhood. *Bone Marrow Transplant* 1998;**21**:287-90.

Thomas-Teinturier C, El Fayech C, Oberlin O, Pacquement H, Haddy N, Labbe M, Veres C, Guibout C, Diallo I and De Vathaire F. Age at menopause and its influencing factors in a cohort of survivors of childhood cancer: earlier but rarely premature. *Hum Reprod* 2013;**28**:488-95.

Utriainen P, Suominen A, Makitie O and Jahnukainen K. Gonadal Failure Is Common in Long-Term Survivors of Childhood High-Risk Neuroblastoma Treated With High-Dose Chemotherapy and Autologous Stem Cell Rescue. *Front Endocrinol (Lausanne)* 2019;**10**:555.

von Ungern-Sternberg BS, Boda K, Chambers NA, Rebmann C, Johnson C, Sly PD and Habre W. Risk assessment for respiratory complications in paediatric anaesthesia: a prospective cohort study. *Lancet* 2010;**376**:773-83.

von Wolff M, Stute P and Fluck C. Autologous transplantation of cryopreserved ovarian tissue to induce puberty-the endocrinologists' view. *Eur J Pediatr* 2016;**175**:2007-2010.

Wallace WH, Anderson R and Baird D. Preservation of fertility in young women treated for cancer. *Lancet Oncol* 2004;**5**:269-70.

Westergaard CG, Byskov AG and Andersen CY. Morphometric characteristics of the primordial to primary follicle transition in the human ovary in relation to age. *Hum Reprod* 2007;**22**:2225-31.

Xu J, Bernuci MP, Lawson MS, Yeoman RR, Fisher TE, Zelinski MB and Stouffer RL. Survival, growth, and maturation of secondary follicles from prepubertal, young, and older adult rhesus monkeys during encapsulated three-dimensional culture: effects of gonadotropins and insulin. *Reproduction* 2010;**140**:685-97.

Yang S, He X, Niu Y, Hildebrandt TB, Jewgenow K, Goeritz F, Tang X, Chang Y, Zhou Q and Ji W. Ovarian response to gonadotropin stimulation in juvenile rhesus monkeys. *Theriogenology* 2009;**72**:243-50.