Sperm DNA Damage in Cancer Patients

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Abstract

Fertility is a growing healthcare issue for a rising number of cancer survivors. In men, cancer itself and its treatment can negatively affect spermatogenesis by targeting the dividing spermatogonia and their cellular environment, ultimately leading to a reduction of testicular germ cells and sperm count. Experimental data and prospective longitudinal studies have shown that sperm production can recover after cancer treatment. But despite this, yet unpredictable, recovery in sperm production, cancer survivors are more at risk to produce sperm with aneuploidy, DNA damage, abnormal chromatin structure, and epigenetic defects even 2 years post-treatment. Sperm DNA alteration is of clinical concern, as these patients may father children or seek assisted reproduction technologies (ART) using gametes with damaged genome that could result in adverse progeny outcomes. Interestingly, large cohort studies revealed lower birth rate but no significant impact on the health of the children born from male cancer survivors (naturally or using ART). Nevertheless, a better understanding of how cocktail of chemotherapy and new anticancer agents affect spermatogenesis and sperm quality is needed to reduce side effects. Moreover, developing new fertility preservation strategies is essential as sperm cryopreservation before treatment is currently the only option but does not apply for prepubertal/young postpubertal patients.

Keywords

Cancer · Chemotherapy · Fertility · Progeny · Radiotherapy · Sperm chromatin · Sperm DNA · Sperm epigenome

Abbreviations

ART	Assisted reproductive technologies
CED	Cyclophosphamide equivalent dose
DFI	DNA fragmentation index
HDS	High DNA stainability
SCSA	Sperm chromatin structure assay
SSCs	Spermatogonial stem cells

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Introduction

Thanks to advances in the medical management of cancer, including early diagnosis and the development of combination chemotherapy treatments, survival rates have increased significantly (Kaatsch 2010; Torre et al. 2016). The number of cancer survivors is therefore constantly increasing, and their quality of life is becoming a major public health issue. Many of the treatments' side effects associated with the diagnosis of cancer have been described (Diller et al. 2009; Miller et al. 2016). A negative impact on reproductive health is part of these long-term effects and can affect the family plans of cancer survivors diagnosed and treated during childhood or at childbearing age. In men in particular, cancer treatments target spermatogenesis (Ragheb and Sabanegh 2010), and cancer survivors have more difficulty to become father (Hohmann et al. 2011; Tang et al. 2016; Yonemoto et al. 2009). Fertility can therefore be a major concern for cancer survivors, especially since 80% of these patients want to have genetically related children and think that their cancer experience will make them better parents (Reinmuth et al. 2008; Schover et al. 2002). It is important to note that data from the Childhood Cancer Survivor Study (CCSS), which uses the largest and most thoroughly characterized cohort of cancer survivors diagnosed during childhood, showed that 53.6% of pediatric cancer male survivors had been medically evaluated for infertility in comparison to 21.4% of siblings (Wasilewski-Masker et al. 2014). In addition, studies on the fertility of cancer survivors show a significant increase in the use of assisted procreation techniques (ART), regardless of age at diagnosis (Stahl et al. 2011; Stensheim et al. 2013). Unfortunately, less than half of survivors report having been informed on this subject during their diagnosis or at the end of treatment (Cherven et al. 2015). The regret of not being adequately informed about the potential risks of anticancer treatments on fertility was also raised predominantly in a focus group study with male survivors and their parents (Stein et al. 2014).

Germ cells are targets of anticancer drugs because of their high cell division activity (Meistrich et al. 1982). Treatments therefore almost always result in low sperm count (Meistrich 1986), and this depletion may be transient or permanent (Schilsky et al. 1980). Determining the risk of infertility after cancer remission remains complex because a combination of factors must be taken into consideration: the type of cancer, the fertility status at the time of diagnosis, the age at diagnosis, the dose, and the combination of treatments received but also probably genetic factors (Jaffe et al. 1988; Müller et al. 1988). Moreover, spermatogenesis recovery with an increase in the number of spermatozoa in the ejaculate might not necessarily be a guarantee of spermatic quality. In this chapter, evidences that motility, morphology, aneuploidy, quality of DNA, and chromatin as well as epigenetic marks can be altered in sperm from cancer survivors will be reviewed. These abnormalities might originate from the cancer itself, but it can also be caused by the treatment. We will also consider the possible consequences of these spermatic abnormalities on the fertility of cancer survivors as well as the health of their offspring, leading to a review of fertility preservation strategies for cancer survivors.

Impact of Cancer on Sperm

The consequences of cancer on spermatogenesis can be assessed in postpubertal patients, on the production of ejaculated spermatozoa. However, a recent study using testicular biopsies taken from prepubertal boys at the time of cancer diagnosis and before treatment gives us the first hint of the effect of childhood cancer on spermatogenesis (Stukenborg et al. 2018). Despite a great variability in the number of spermatogonia in the 12 biopsies collected, the study suggested that genetic abnormalities in hematological diseases (thalassemia majors, Fanconi anemia, and immunodeficiency caused by variant of FOXP3-gene) may be associated with reduced numbers of spermatogonia (Stukenborg et al. 2018). As spermatogonia fuel continuous sperm production

later in adulthood, a reduction in their number in the prepubertal testis could likely result in a low sperm count. In parallel, in pubertal men, it has been shown that the cancer itself can affect sperm production, and this depends on the nature of the cancer and its stage (O'Flaherty et al. 2008; Rueffer et al. 2001). For example, in the case of leukemia or lymphoma, studies have shown that the stage of the disease is positively correlated with the negative impact on spermatogenesis (Rueffer et al. 2001; Gandini et al. 2003). Also, Hodgkin's lymphoma seems to greatly affect fertility, since in the Rueffer et al. study (Rueffer et al. 2001), 70% of the 158 men, tested before starting anticancer treatments, have at least 1 altered sperm parameter compared to the World Health Organization (WHO) standards (Cooper et al. 2010). In the case of testicular cancer, sperm concentration, motility, and morphology are significantly decreased compared to controls which could be due to a predisposition to testicular cell-related pathologies (O'Flaherty et al. 2008).

Chromatin structure abnormalities, DNA breaks, and increased frequency of aneuploidy in spermatozoa can also be measured upstream of cancer treatments compared to controls. Besides, it has been shown that the integrity of sperm DNA is affected by cancer, regardless of the nature of the cancer tested (Bujan et al. 2014, 2013; Kumar et al. 2018; Martinez et al. 2017; Meseguer et al. 2008; O'Flaherty et al. 2010, 2008; Paoli et al. 2015; Stahl et al. 2009; Tamburrino et al. 2017; Tempest et al. 2008). For example, using the COMET assay or the chromatin dispersion test on sperm collected before treatment, from groups of 6 to 26 men diagnosed with various cancers, it has been shown that the fragmentation of sperm DNA is significantly increased compared to the control group (Meseguer et al. 2008; O'Flaherty et al. 2008). More specifically, sperm chromatin structure assay (SCSA) analysis on sperm from men diagnosed with testicular cancer shows a percentage of DNA fragmentation index (DFI), higher than the controls and comparable to a group of infertile men (O'Flaherty et al. 2008; Stahl et al. 2009). In addition, the COMET assay revealed a higher rate of sperm DNA breaks in men diagnosed with testicular cancer or Hodgkin's lymphoma compared to community controls (O'Flaherty et al. 2008). Finally, chromatin compaction, measured by chromomycin A3 labelling, is altered in sperm from men diagnosed with Hodgkin's lymphoma (O'Flaherty et al. 2008). Potential mechanisms of cancer-induced sperm DNA damage may include genetic mutations, changes in hormone levels, fever, inflammation, or general oxidative stress.

Cancer can therefore affect not only the quantity but also the quality of sperm. But it should be noted that, at the individual level, the analysis of chromatin integrity using the SCSA before treatment cannot predict the chances of producing intact spermatozoa after cancer remission (Fossa et al. 1997). Indeed, cancer treatments can also be harmful to sperm production and sperm integrity.

Impact of Cancer Treatment on Sperm

Radiotherapy

The risks of radiation and radiation therapy on male reproductive health through their negative impact on semen parameters have been intensely studied for decades. Various preclinical and clinical studies have indicated the effect of radiation on reduction in the number of type A spermatogonia leading to impairment in spermatogenesis output (Meistrich 2013). It has been estimated that after a single dose of ~10 Gy, only ~15% of patients recover sperm count (Jacob et al. 1998; Sanders et al. 1996). Further, fractionated radiation may be more damaging than a single dose as the former causes greater delays in spermatogenic recovery with lower total doses required to cause permanent azoospermia (Abuelhija et al. 2013; Sandeman 1966).

In addition to its effects on conventional semen parameters, the impact of radiation on sperm DNA damage is of more clinical concern, as these patients may seek ART using gametes with potentially damaged genome for reproduction that could result in adverse reproductive

outcomes. Various groups of investigators have evaluated the impact of radiation on sperm DNA integrity. However, few have included analyses in patients receiving only radiation therapy without chemotherapy, which, as seen in the next section of this chapter, can also have profound effect on sperm chromatin integrity. Paoli et al. reported that for men with testicular seminoma receiving a dose of radiotherapy around 2550 cGy, there is a rise of DFI at 3 and 6 months post-treatment (Paoli et al. 2015). No significant increases, however, were noted at 9, 12, and 24 months post-treatment (Paoli et al. 2015). This transient increase in DFI was also reported in two prospective longitudinal studies on testicular cancer patients treated with adjuvant radiotherapy (Bujan et al. 2013; Stahl et al. 2004). The effects of radiotherapy on the fraction of highly DNA-stainable (HDS) cells among men treated for testicular cancer were less consistent. Some investigators reported a significant decrease in HDS sperm, indicating improved chromatin condensation, with increasing time at 9, 12, and 24 months since the end of treatment (Paoli et al. 2015). Others reported a transient increase of HDS sperm but no subsequent improvement in sperm chromatin condensation (Bujan et al. 2013). Interestingly, the one study on childhood cancer survivors treated with radiotherapy only showed that sperm exhibited statistically significantly higher DFI than the controls (Romerius et al. 2010). The odds ratio (OR) for having DFI >20% in this group was high (OR, 4.9; 95% CI, 1.3–18), but DFI was not associated with dose of scattered testicular irradiation (Romerius et al. 2010).

Several observations have been reported that may illustrate the potential mechanisms of radiation-induced sperm DNA damage and its impact on the sperm genome and epigenome. In a retrospective analysis, Kumar et al. reported altered sperm chromatin integrity in radiation health workers is associated with increase in seminal plasma antioxidant level, probably an adaptive measure to tackle the oxidative stress to protect sperm genomic and functional integrity in exposed subjects (Kumar et al. 2014). In another study, the same group of investigators reported elevated global hypermethylation of spermatozoa (Kumar et al. 2013). Further prospective controlled analyses on a wider scope of cancer diagnoses that can benefit from radiation therapy are still required to fully illustrate the impacts of radiation on the temporal changes of sperm genomic and epigenomic integrity, actual reproductive risks, and developmental health of the offspring, particularly in the context of using ART.

Chemotherapy

Chemotherapy regimens may include cocktails of alkylating agents, antimetabolites, and antitumor metabolites that specifically target proliferating cells. Most of these chemotherapeutic agents are known to disrupt spermatogenesis and male germ cell function as they target actively dividing spermatogonia leading to decreased sperm counts (Meistrich 2013). The extent of injury post-chemotherapy depends on the dosage, duration, and type of agents used (Meistrich 2013).

The first evidence of alteration of spermatogenesis by chemotherapies was observed postmortem on histological sections in 27 out of 30 men treated with nitrogen mustard treatments (Spitz 1948). Since then, alkylating agents have been shown to be the most harmful to male fertility (Meistrich 2013). The use of high-dose alkylating agent treatments results in a high cancer cure rate, but in return, men are at higher risk of developing definitive azoospermia (van der Kaaij et al. 2007; Viviani et al. 1985; Pryzant et al. 1993; Paoli et al. 2016; Sanders et al. 1996). In fact, the chances of finding a sperm concentration in WHO standards after remission are reduced by 33% when cyclophosphamide is administered at dose greater than 9.5 g/m^2 (Pryzant et al. 1993). In addition, the administration of alkylating agents, in comparison with chemotherapy without alkylating agents, reduces by 30% the chances of having a follicle-stimulating hormone (FSH) concentration in the standards, FSH levels being inversely proportional to the production of spermatozoa (Gordetsky et al. 2012; van der Kaaij et al. 2007). The negative impact of alkylating

agents has been shown to be dose-dependent, but the nature of the molecule received may also alter the impact on spermatogenesis. For example, compared to cyclophosphamide, procarbazine is already harmful to sperm production at doses lower than 9.5 g/m^2 (Meistrich 2013). Thus, when the dose of alkylating agent is reduced (cyclophosphamide equivalent dose (CED) <10 g/m^2), the chances of recovery of spermatogenesis after cancer remission are greater but still remain unpredictable (Pryzant et al. 1993; Green et al. 2014). In the case of pediatric cancers as well, a CED of 4000 mg/m^2 is associated with a decreased number of spermatogonia (counted in testicular biopsies) and decreased sperm count among survivors (Green et al. 2014; Chow et al. 2016; Poganitsch-Korhonen et al. 2017; Stukenborg et al. 2018).

Today, chemotherapy protocols include some restrictions to limit side effects (Pritchard-Jones et al. 2013). Therefore, when possible, the use of alkylating compounds, known for their long-term harmful systemic effects, is reduced. In addition to lower toxicity, protocols are in favor of mixing the use of several drugs at lower doses (Pinto et al. 2011). However, in vitro studies on spermatogonial cells showed that combination of drugs induced higher toxicity than each drug alone (Beaud et al. 2017b; Marcon et al. 2010). Likewise, even a mixture without alkylating agents commonly used in hematologic cancer treatment (doxorubicin plus vincristine) could induced spermatogonial cell death at clinically relevant doses (Beaud et al. 2017b). Notably, such toxicity data on the male germ line are not available for each commonly used chemotherapeutic compound, and the impact of chemotherapy cocktails remains largely unknown. Using animal models, we have shown that adult male rats exposed to the combination of chemotherapeutic agents used to treat testicular cancer (bleomycin-etoposide-cisplatin: BEP regimen) or non-Hodgkin's lymphoma (cyclophosphamide-doxorubicin-vincristine-prednisone: CHOP regimen) have a significant decrease in sperm count but also an increased amount of DNA breaks in spermatozoa, leading to impaired fertility and adverse progeny outcome (Bieber et al. 2006; Delbes et al. 2009, 2007; Vaisheva et al. 2007). Although sperm production returned to control values after a recovery period, DNA damage persisted, suggesting impaired DNA repair ability in male germ cells (Delbes et al. 2010).

Similarly, prospective longitudinal studies on cancer survivors of reproductive age, treated with cocktails of chemotherapy for testicular cancer or various types of lymphoma, demonstrated that cancer treatment negatively affected sperm production in all cancer survivors (Bujan et al. 2014, 2013; O'Flaherty et al. 2010, 2012; Smit et al. 2010). The azoo- or oligozoospermia induced can be temporary or permanent (Chan 2009). Despite a possible recovery of spermatogenesis for some patients, as demonstrated by an increase in semen sperm density and motility 1 or 2 years post-chemotherapy, sperm DNA damage can carry on. More specifically, using the SCSA and the COMET or the TUNEL assay, 3–6 months post-chemotherapy, it was shown that the DFI and DNA breaks were statistically higher in sperm from survivors of testicular cancer or lymphoma than in a control group (Bujan et al. 2014, 2013; O'Flaherty et al. 2010, 2012; Paoli et al. 2015). For testicular cancer patients, 1 year post-treatment, DFI and DNA breaks returned to control values (Bujan et al. 2013; O'Flaherty et al. 2010, 2012; Paoli et al. 2015), but chromatin compaction measured by HDS remained altered even 2 years after post-treatment (O'Flaherty et al. 2012). Interestingly, survivors of lymphoma displayed high DFI even 2 years post-chemotherapy, but this was not associated with DNA breaks or abnormal chromatin compaction (Bujan et al. 2014). These data suggest that depending on the cocktail of chemotherapy administered, long-term consequences on sperm quality could vary. Mechanisms of chemotherapy-induced sperm DNA damage are yet unclear but may be the consequences of impaired DNA repair (Delbes et al. 2010) or changes in the expression of genes involved in chromatin remodelling during spermiogenesis (Maselli et al. 2013).

In parallel, an increased incidence of aneuploidy has also been measured in sperm of cancer survivors up to 6 months post-treatment

(Tempest et al. 2008; Rives et al. 2017). This might be due to the mutagenic impact of chemotherapies. Most studies show that sperm aneuploidy levels may return to values similar to those measured before treatment or similar to the control group within 1 or 2 years post-treatment (Thomas et al. 2004; Tempest et al. 2008; Martinez et al. 2017). However, the return to a basal level of aneuploidy depends on the treatment administered (Martinez et al. 2017). In fact, lymphoma survivors treated with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) have a higher chance to display control levels of sperm aneuploidy 1 year post-treatment than those treated with CHOP or MOPP-ABV (MOPP: mechlorethamine, oncovin, procarbazine, prednisone) who still displayed high level of aneuploidy even 2 years post-chemotherapy (Martinez et al. 2017).

Epigenetic marks could also be affected in sperm from cancer survivors. Experimental data are available showing that 9-week treatment of BEP in adult male rat induced changes in sperm DNA methylation profiles (Chan et al. 2012) and histone distribution patterns (Bagheri-Sereshki et al. 2016; Maselli et al. 2012, 2013). In humans, only two studies have examined DNA methylation in sperm after chemotherapy treatment. On the one hand, a case study demonstrates a 10% progressive loss of methylation of the H19 paternal imprinted gene up to 5 months after temozolomide treatment (Berthaut et al. 2013). On the other hand, immunoprecipitation analysis of methylated DNA followed by high-throughput sequencing revealed several differently methylated regions in sperm from pediatric cancer survivors treated with cisplatin, compared to control spermatozoa (Shnorhavorian et al. 2017). The importance of altered epigenetic marks in the germ line is still unclear, but recent data suggest that the sperm epigenome can affect embryo development and the health of future generations (Wu et al. 2015). As well it has been suggested as a mechanism for transgenerational transmission. Better understanding the effects of anticancer drugs on the germ line epigenome is therefore very relevant and even more with the development of drugs targeting epigenetic pathways to cure cancer.

It is important to note that these data showing the long-term damage in sperm DNA and chromatin structure have been generated on postpubertal individuals, while studies are lacking on the long-term impact of prepubertal chemotherapy on sperm chromatin quality. Although the prepubertal testis does not produce mature spermatozoa, it does contain diploid spermatogonia from which haploid spermatozoa will be derived. Little data using prepubertal animal models to elucidate mechanisms of action are available. Mainly, exposures to single agents such as doxorubicin, etoposide, or cisplatin have been shown to primarily deplete the testis of germ cells and to have a long-term impact on Sertoli cells (Brilhante et al. 2012; Lirdi et al. 2008; Okada et al. 2009). Importantly, Vendramini et al. have shown that doxorubicin-exposed rat spermatogonia in prepubertal rats produced long-term damage to sperm DNA and that this might be the cause of compromised conceptus development and reduced pregnancy outcome (Vendramini et al. 2012). In humans, only two studies investigated DNA breaks and/or chromatin integrity in sperm from childhood cancer survivors, years after chemotherapy and/or radiotherapy ended (Romerius et al. 2010; Thomson et al. 2002). In their study of 33 childhood cancer survivors, Thomson et al. did not observe any difference in sperm DNA integrity measured by the TUNEL assay when compared to age-matched controls (Thomson et al. 2002). On the other hand, Romerius et al. used the SCSA and studied 99 childhood cancer survivors for whom they observed an increased DFI compared to aged-matched controls which was of borderline statistical significance (Romerius et al. 2010). Both studies grouped childhood cancer survivors with various diagnostics, heterogeneous treatments, and a range of age at diagnosis. Therefore, analyses were done without segregating the impact of prepubertal and postpubertal treatment. While it was thought that being prepubertal during anticancer therapy conferred protection against gonadal damage, more recent evidence of the impact on long-term sperm production has led some researchers to conclude that the prepubertal gonad is even more vulnerable to the cytotoxic

effects of chemotherapy than the adult testis (Revel and Revel-Vilk 2008). Our most recent data focused on survivors of childhood hematologic cancer (Beaud et al. 2017a). Although limited by the number of subjects (6 prepubertal and 7 post-pubertal survivors), the data indicate that, independently of the age of diagnosis, childhood cancer survivors have a higher risk of no or low sperm count, and when sperm are present, chances of DNA and chromatin abnormalities appear similar to those seen in the general population. Nevertheless, exposure to anthracyclines, and doxorubicin in particular, could have long-term consequences on sperm integrity (Beaud et al. 2017a). According to current knowledge, the importance of age at diagnosis in relation to puberty on potential long-term effect on sperm DNA and chromatin remains poorly understood.

Impact on Fertility and the Health of Progeny

Because cancer and its treatment can affect the DNA, chromatin, and epigenome of survivors' sperm, it is important to know if this ultimately affects their fertility and/or the health of their progeny (Tremblay et al. 2017). Indeed, preclinical studies suggested that sperm DNA fragmentation induced by testicular irradiation may result in a variety of checkpoint responses in early embryo development and transgenerational genomic instability in the offspring (Adiga et al. 2007, 2010; Shiraishi et al. 2002). Moreover, paternal exposure to genotoxic agents or endocrine disruptors can induce genetic or epigenetic mutations in gametes which can negatively impact the health of the offspring, even over several generations (Danchin et al. 2011; van Otterdijk and Michels 2016; Xin et al. 2015).

The most important impact of cancer and its treatment is the decrease in sperm count that can be permanent and is most probably due to the toxicity of the drugs on spermatogonia (Meistrich 2013; Tremblay et al. 2017). Another significant consequence is the decrease in birth rate that has been reported in three large cohort studies combining all types of cancers and compared to the general population (Chow et al. 2016; Green et al. 2003; Tang et al. 2016). This is in agreement with the experimental data, and these effects depend on the type of treatment and the dose received (Chow et al. 2016). Beyond the difficulty of conceiving, the cancer history does not seem to have a significant impact on the health of the offspring. Some evidences show a slight increase in the risk of congenital anomalies (Seppanen et al. 2016; Stahl et al. 2011), but this risk remains close to that of the general population and seems to improve with the evolution of cancer treatments (Seppanen et al. 2016). As cancer survivors are more likely to use ART (Stensheim et al. 2013), it is important to note that the large cohort studies evaluated children born from ART separately and did not observe a higher risk of congenital abnormality for children conceived by in vitro fertilization or intracytoplasmic sperm injection (Seppanen et al. 2016; Stahl et al. 2011).

In parallel, the risk of de novo mutations, chromosomal abnormalities, or cancer development in children of male cancer survivors does not appear to be higher than in the general population, after adjusting for family heredity (Byrne et al. 1998; Kryukov et al. 2016; Winther et al. 2004; Hawkins et al. 1995). Even whole genome sequencing on families of two testicular cancer survivors did not show any genetic impact due to treatment (Kryukov et al. 2016). Finally, there does not appear to be a significant increase in the frequency of postnatal mortality among children of men with a history of cancer (Dere et al. 2013; Dufour et al. 2010; Tang et al. 2016). In addition, it has been shown that hospitalization rates for children of survivors up to the age of 15 are not higher than in the general population once heredity factors are removed (Winther et al. 2010). The study of 126,696 individuals born in Sweden of men with a history of cancer did not show a higher mortality rate in this population, regardless of when they were born in relation to the diagnosis of cancer, the type of cancer, or the age of the diagnosis of their father (Tang et al. 2016).

Therefore, because of the very low risk for the health of the progeny, the American Society for Reproductive Medicine Ethics Committee

considers that for patients who experience gonadotoxic therapy, concerns about the well-being of descendants are not sufficient to refuse them assistance for reproduction (NTP Monograph: Developmental Effects and Pregnancy Outcomes Associated With Cancer Chemotherapy Use During Pregnancy 2013). However, the scope of the epidemiological data remains limited in the measured health parameters, in addition to being restricted to the first generation. In order to know if there are transgenerational effects in the human population, it would be necessary to pursue cohort studies over several generations. The demonstration of epigenetic changes in the sperm of treated fathers could constitute a transgenerational transmission mechanism (Berthaut et al. 2013; Shnorhavorian et al. 2017).

Fertility Preservation Strategies

While spermatogenesis may recover in some cancer survivors over time, it is currently impossible to predict for whom and when it will resume. Sperm banking by cryopreservation prior to cytotoxic cancer treatment is therefore the best and currently the only feasible option for fertility preservation (Chan and Robaire 2011; Wallace et al. 2005a). However, when anticancer treatment does not cause infertility, there is still a debate about the use of cryopreserved sperm over fresh semen (Vakalopoulos et al. 2015). Indeed, a few years after the end of treatment, sperm DNA and chromatin integrity may be better than before treatment, at the time of banking, when it might have been affected by the disease (Paoli et al. 2016). In addition, sperm cryopreservation can induce oxidative stress in spermatozoa and cause DNA damage (Thomson et al. 2009; Lusignan et al. 2018). Sperm analysis including sperm DNA and chromatin integrity assays could be recommended before cryopreservation and after cancer recovery to assess which sperm to use. Moreover, we and others have developed clinically reliable strategies for sperm selection using magnetic-activated cell sorting (MACS) with annexin V to eliminate sperm that show apoptotic features associated with altered chromatin. The addition of annexin V-MACS to routine sperm preparation in the clinic has been shown to be efficient in enriching samples with high motility, viable, and non-apoptotic spermatozoa (Delbes et al. 2013; Said and Land 2011) with intact chromatin and DNA (Delbes et al. 2013; Tavalaee et al. 2012) and high fertilization potential (Lee et al. 2010). Such a strategy led to a successful pregnancy and live birth in a couple with recurrent ART failure, using cryopreserved sperm from a cancer patient survivor (Herrero et al. 2013).

Unfortunately, the sperm banking option only applies to pubertal patients whose sperm production is ongoing and who are able to provide a sperm sample (Chan and Robaire 2011). In addition, even young, newly pubescent boys (14–19 years old) cannot always provide a sperm sample, and when they can, the sperm is often of poor quality in terms of concentration, volume, or motility (Postovsky et al. 2003). In fact, for preadolescent boys with cancer, no clinically proven methods are available to preserve fertility. However, some centers do offer experimental protocols, such as testicular tissue cryopreservation before treatment, with the hope that the unexposed germ cells present in these biopsies can be used for future reproduction (Trost and Brannigan 2012). Indeed, transplantation of spermatogonial stem cells (SSCs) isolated from testicular biopsies has been proven efficient first in mice and more recently in primates and has therefore been proposed as a promising strategy to restore male fertility for cancer survivors (Wallace et al. 2005b; Mitchell et al. 2009; Chan and Robaire 2011; Goossens and Tournaye 2013; Struijk et al. 2013; Anderson et al. 2015; Jahnukainen et al. 2015; Raffoul et al. 2016; Brinster 2002; Hermann et al. 2012). In this procedure, testicular tissue removal is a relatively minor surgery, but it remains invasive and requires general anesthesia (Raffoul et al. 2016; Gupta et al. 2016). The biopsy is then frozen for later use in the patient's life. Freezing protocols are promising in humans (Keros et al. 2007) but

still require a better characterization of stem cell functionality after thawing (Anderson et al. 2015). Testicular biopsy programs for pediatric patients already exist, in view of future developments in this field (Sadri-Ardekani et al. 2016). Afterward, from the thawed testicular tissue, three main protocols are being investigated, although none are currently approved in humans:

1. Self-transplantation of testicular tissue once the patient is cured and reached adulthood, to allow the immature tissue to produce sperm and restore fertility (Mitchell et al. 2009; Chan and Robaire 2011). This technique is effective in mice but remains to be tested in humans (Anderson et al. 2015). In addition, biopsies carry a risk of contamination by cancer cells, especially in the case of blood cancers, and thus the reintroduction of cancer in survivors (Jahnukainen et al. 2015).
2. Self-transplantation of SSCs isolated from the testicular parenchyma, purified and amplified in vitro (Struijk et al. 2013), in order to colonize the seminiferous tubules and initiate spermatogenesis in the adult patient (Struijk et al. 2013). Again, there are several technical limitations such as effective purification of SSC to prevent reintroduction of cancer cells (Struijk et al. 2013; Goossens and Tournaye 2013; Jahnukainen et al. 2015).
3. Sperm maturation in vitro from isolated SSC and use in ART (Jahnukainen et al. 2015).

In 2016, Perrard et al. successfully produced sperm-like cells from fresh or frozen biopsies of men whose spermatogenesis was inhibited (Perrard et al. 2016). Although this option addresses the issue of reintroduction of cancer, it has not yet been tested with human prepubertal tissue.

In parallel, another interesting strategy for fertility preservation includes the improvement of existing protocols used in anticancer therapies to provide protection to the healthy cells. For example, inactivating spermatogenesis by suppression of gonadotropins using a GnRH antagonist during treatment has been investigated, but unfortunately, not only clinical trials have so far not shown a convincing level of benefit (Meistrich and Shetty 2008); it might be ineffective for prepubertal children as the proliferation of germ cells in prepubertal primates appears to be gonadotropin-independent (Kelnar et al. 2002). Co-treatment with radioprotectants has been effective in cases of cancer related to aging, to reduce the side effects in some organs without reducing the effectiveness of treatment against cancer (Gómez et al. 2013; Kemp et al. 1996). For example, co-treatment protocols are now included in oncology clinical practice guidelines to reduce neurotoxicity (Hershman et al. 2014). Radioprotective compounds have been suggested to exert protective action through their antioxidant properties and by increasing DNA repair capacity (Cabral et al. 2014; Lirdi et al. 2008). Data using a prepubertal rat model showed that carnitine and amifostine could be efficient in maintaining male germ cells against the cytotoxic impact of cisplatin (Lirdi et al. 2008), etoposide, or doxorubicin (Cabral et al. 2014; Okada et al. 2009). On one hand, carnitine pretreatment maintained sperm DNA integrity, embryo implantation rate, and litter size despite doxorubicin treatment (Cabral et al. 2018). On the other hand, amifostine pretreatment actually increased sperm DNA breaks and abnormal chromatin structure measured by the COMET assay and the SCSA, respectively, probably increasing embryonic loss rate (Vendramini et al. 2012). Although promising, these in situ protection methods still remain experimental, and the risks of the radioprotectant having negative impact on its own currently outweigh the potential benefit.

Overall, novel fertility preservation strategies, developed by evidence-based research, are urgently needed not only to help male cancer patients to preserve fertility but also to help reduce the risks of long-term adverse reproductive outcomes on sperm quality. This would in term improve the quality of life of many boys and men affected by cancer.

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