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Review

Impacts of cancer therapy on male fertility: Past and present

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ABSTRACT

Over the past two decades, advances in cancer therapy have significantly improved survival rates, particularly in childhood cancers. Still, many treatments pose a substantial risk for diminishing future fertility potential due to the gonadotoxic nature of many cancer regimens, justifying fertility preservation programs for both childhood and adult cancer patients. To assure a balance between offering fertility preservation and actual chance of infertility post-treatment, guidelines are in place. However, assessing the actual risk of infertility after treatment remains challenging, given the multi-faceted approach of many cancer treatment plans, which are continuously evolving. This review discusses the evolution of cancer therapy over the past 20 years and attempts to assess their impact on fertility after treatment. Overall, cancer regimens have shifted from broadly killing fast dividing cells to more targeting therapies, reducing collateral damage in general. Although progress has been made to reduce overall toxicity, unfortunately this does not automatically translate to reduced gonadotoxicity. Therefore, current fertility preservation programs continue to be an important part of cancer care.

1. Introduction

When a person is diagnosed with cancer, not only do they face burdensome cancer therapy, but also the threat of future infertility due to gonadotoxicity of many anti-cancer regimens (Wallace et al., 2005). For males, the cause of cancer therapy associated infertility relates to the sensitivity of spermatogonia to anti-cancer regimens, which usually include chemotherapy and/or radiotherapy (van der Meer et al., 1992a; Meistrich 2013, Zheng et al., 2018). As spermatogonia, and especially spermatogonial stem cells (SSCs), form the basis of life-long spermatogenesis, destroying this population will cause irreversible infertility. The risk that different treatment regimens pose to future fertility varies from low to very high, and is determined by the treatment modalities used, combinations, and dosage (Relander et al., 2000; Brougham et al., 2003). It is also important to consider the patient's age and pubertal status at time of diagnosis, as well as the potential impact of the disease itself (Masliukaite et al., 2023).

Cancer regimens over the past 20 years have evolved, resulting in improved survival. For childhood cancers, the five-year survival rate is currently 81% (Botta et al., 2022). Given this success, late effects of both disease and treatment are becoming increasingly important. In particular, patients treated with higher doses of alkylating chemotherapy or radiotherapy are at significant risk of fertility problems in the future (Byrne et al., 2004; Jahnukainen et al., 2011; Poganitsch-Korhonen et al., 2017).

To safeguard the patient's ability to start a family after cancer,

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clinical fertility preservation programs are in place worldwide. Cryopreservation of sperm before cancer treatment is the most effective procedure to preserve fertility in adult men and is an established procedure that should be offered as standard practice. If the semen is devoid of sperm, an onco-testicular sperm extraction (onco-TESE) has been successful for retrieving sperm in 50% of patients (Micol et al., 2022; Ogouma et al., 2022). Cryopreserved sperm obtained from semen or surgical extraction can be used for a variety of fertility treatments, including intra-cervical insemination, intra-uterine insemination (IUI) and in-vitro fertilization (IVF), depending on the quality and quantity of available sperm. Still, in some adult cases no sperm can be retrieved.

For pre-pubertal boys, cryopreservation of sperm is not yet possible, as spermatogenesis has not been initiated. Cryopreservation of a testicular biopsy prior to cancer treatment may provide an option to preserve their fertility. Through this procedure, tissue containing SSCs that have not been exposed to potentially gonadotoxic treatments can be stored; while this procedure remains experimental at present, the aim is that tissue will be used in the future to restore fertility (Ginsberg et al., 2010; Goossens et al., 2020; Duffin et al., 2024).

Because such procedures in pre-pubertal and adolescent boys are experimental at present, testicular biopsy for these patients should only be considered when there is a high risk of future infertility post-treatment (Mulder et al., 2021). However, estimating future risk of infertility is very difficult and imprecise, and often relies on assessing long-term follow-up patients, who may have received cancer treatments that have been superseded. Certain treatment modalities, such as total body irradiation (TBI), testicular irradiation, and high dose alkylating chemotherapy, are widely accepted to pose a high risk to future fertility. Calculation of the cyclophosphamide equivalent dose (CED) can be used to estimate the risk of alkylating agent-containing treatment regimens (Green et al., 2014); however, variations exist in the CED thresholds used to assess eligibility for fertility preservation (Green et al., 2014; Duffin et al., 2024). Additionally, there are classes of chemotherapy agents which have been demonstrated to affect male fertility, such as platinum-based agents, which are not currently incorporated into the CED calculation (Tian En et al., 2020). These challenges in defining risk of treatment to fertility pose a practical problem for pediatric oncologists when counseling their patients and deciding when to offer fertility preservation.

As cancer regimens have advanced in the past 20 years, the actual risk for infertility after cancer treatment may have changed as well. In this review, we will outline the evolution of cancer therapies and how this may impact fertility after treatment (Fig. 1). Assessing risk of treatment-related infertility is challenging, but informing clinicians may help guide fertility discussions and decisions about when fertility preservation may be indicated.

2. Understanding the off-target effects of cancer therapy to the testis

Cancer treatment is often a multi-faceted approach, and may involve surgery, chemotherapy, radiotherapy and, increasingly, immunotherapy. Both chemotherapy and radiotherapy result in cytotoxicity due to various characteristics of cancer cells, which forms the basis of their therapeutic effectiveness. However, due to similarities between cancer cells and germ cells (Bruggeman et al., 2023), the healthy germ cells may also be damaged by such treatment. In this section, we will outline the working mechanisms of cancer treatments and how they affect germ cells.

2.1. Working mechanism of chemo- and radiotherapy

Chemotherapy can be divided into classic DNA-targeting drugs and those that do not target DNA. Among the DNA-targeting drugs are alkylating agents, antimetabolites, cytotoxic antibiotics, and DNA topoisomerase inhibitors; chemotherapy drugs which do not target DNA include antimitotic agents. Other anti-cancer drugs that do not fall within the classification of traditional chemotherapy include targeted therapies, such as hormone therapies, enzyme inhibitors and immunotherapy. In this part, we will primarily focus on the working mechanisms of cytotoxic chemotherapies and radiotherapy.

2.1.1. Alkylating and alkylating-like agents

Alkylating agents are amongst the earliest anticancer drugs to be

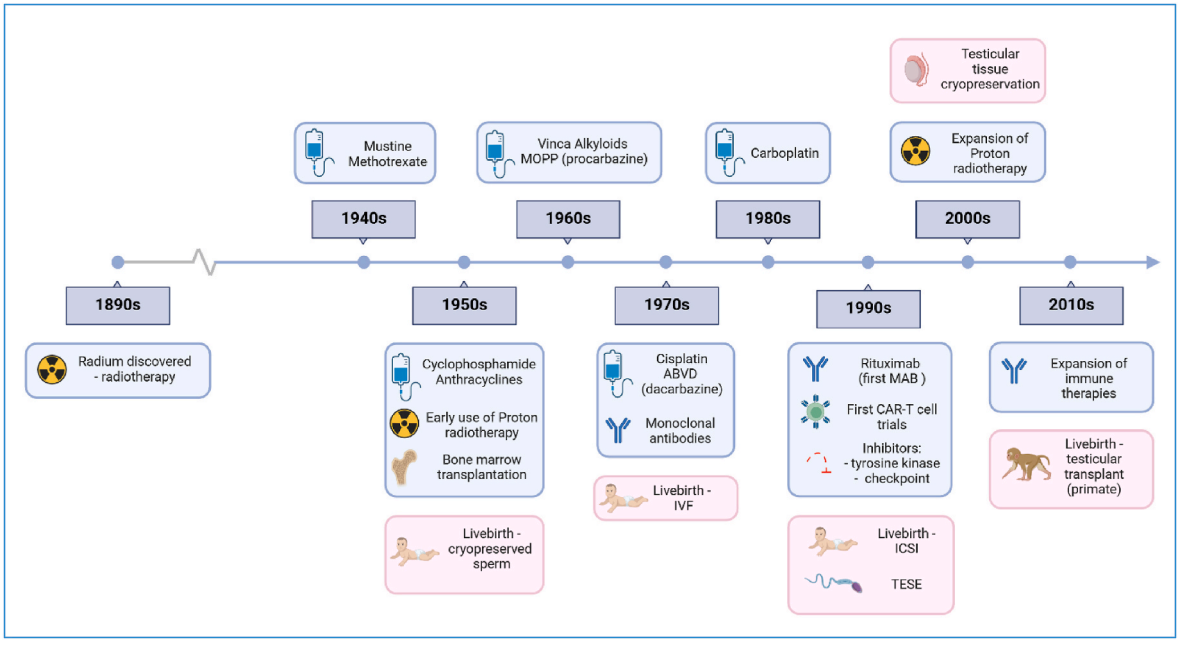

Fig. 1. Timeline of advances in cancer treatment, fertility preservation practices and fertility treatments relevant for males receiving gonadotoxic therapies.
Timeline of advances in cancer treatment (blue boxes) and fertility preservation practices and fertility treatments (red boxes) relevant for males receiving gonadotoxic therapies. MOPP - mustagen, oncovin, procarbazine and prednisone, ABVD - adriamycin, bleomycin, vinblastine, dacarbazine, ICSI - intracytoplasmic sperm injection, IVF - in-vitro fertilisation, MAB - monoclonal antibodies, CAR-T cell - chimeric antigen receptor T cell, TESE - testicular sperm extraction.

discovered (Hall and Tilby 1992). Initially used as a weapon during World War I, mustard gas was found to cause a dramatic reduction in rapidly dividing blood cells in exposed soldiers (Goodman, 1946; DeVita and Chu 2008). Once this property of these agents was recognised, animal studies were conducted which showed that nitrogen mustard was able to induce the regression of lymphoid tumors in mice and subsequently in patients with lymphoma (DeVita and Chu 2008) (Fig. 1). These findings ultimately led to the development of cyclophosphamide, which remains one of the most commonly used chemotherapeutics in children and adults with cancer. Other commonly used alkylating agents include busulfan, melphalan, ifosfamide, and procarbazine.

Alkylating agents have a common mechanism of inducing DNA damage in proliferating cells. The mechanism of action of these agents involves transfer of an alkyl group to nucleotides (mainly guanine) in the DNA. This results in one or more DNA cross links resulting in DNA damage, DNA breaks, inhibition of DNA replication and subsequent cell death (Drablos et al., 2004; Calderon-Montano et al., 2014). This results in the death of cancer cells, but also in non-malignant cells. Cancer cells are genomically unstable and often show defects in DNA repair potential or a failure to stop the cell cycle to allow for repair of DNA damage. However, non-malignant cells have an excellent repair mechanism and can either repair chemotherapy damage or induce apoptosis when damage cannot be repaired before transmitting damaged DNA to a daughter cell. The damage to healthy cells forms the basis of off-target effects and acute and long-term consequences of exposure to alkylating agents.

A related group of drugs are the platinum-based agents, such as cisplatin and carboplatin (Fig. 1). The cytotoxic mechanism of these drugs are generated by binding to the purine bases guanine and adenine, resulting in the formation of DNA inter- and intra-strand crosslinks, as well as platinum-DNA adducts, leading to cell death (Eastman 1985, Fichtinger-Schepman et al., 1987).

2.1.2. Antimetabolites

Antimetabolites, such as methotrexate and cytarabine, are a group of anticancer compounds that inhibit nucleic acid synthesis by inducing the incorporation of altered nucleotides into the DNA or RNA; most of these drugs are cell cycle specific. This class of drugs also includes cytotoxic antibiotics, such as anthracyclines (e.g. doxorubicin), which are non-cell cycle specific (Fig. 1). Anthracyclines act by intercalating between base pairs in the DNA helix, thereby altering structure and function.

2.1.3. Antimitotics

Among other classes of chemotherapeutics that do not intercalate in DNA are antimitotic compounds that interfere with cell cycle. This includes microtubule targeting drugs such as vincristine and vinblastine (Calderon-Montano et al., 2014).

2.1.4. Targeted therapies

In the early 2000s, targeted therapies began to be approved for clinical use; the principle of targeted therapy is that treatments will target specific enzymes or pathways that allow cancer cells to proliferate and survive. One of the first widely used targeted therapies was imatinib, a tyrosine kinase inhibitor which was approved for treatment of chronic myeloid leukaemia (CML) in 2001 (Druker et al., 2001; Cohen et al., 2002). Targeted anti-cancer drugs may inhibit specific enzymes needed for the survival of the cancer cell. As an example, inhibiting proteasomes will lead to degradation of cellular proteins; carfilzomib is an example of a proteasome inhibitor which is used to treat adults with multiple myeloma (Yee 2021).

In recent decades, immunotherapy has emerged as a mainstay of cancer treatment. Broadly speaking, immunotherapy utilises components of the immune system such as cytokines and immune cells to induce anti-cancer effects (Yang 2015). Agents include targeted antibodies, such as blinatumomab, a bispecific antibody which targets both CD19 cancer cells and CD3 T cells, enabling activated T cells to identify and eradicate the cancer cells. Blinatumomab is currently used for treatment of certain patients with acute lymphoblastic leukaemia (Kantarjian et al., 2017). Another example of immunotherapy is the use of chimeric antigen receptor (CAR)-T cell therapy. This is an adaptive cell therapy, whereby patient-derived T cells are genetically modified to directly target tumor-associated antigens (Porter et al., 2011).

2.1.5. Radiotherapy

Radiotherapy targets cancer cells with high-energy radiation, causing DNA damage, preventing cell division and causing their death (Bouwman and Jonkers 2012). This treatment can be administered as photon therapy, using X-rays or gamma rays, or proton therapy, which employs positively charged protons.

Photon radiotherapy delivers radiation that penetrates through the tumor but also includes surrounding tissues, potentially causing side effects in these areas. Protons, however, release their maximum energy at a specific depth, directly at the tumor site, and therefore have no 'exit dose', thereby sparing nearby healthy tissues. This more recent therapy (Fig. 1) may result in a reduction in adverse long-term effects of radiotherapy to neighbouring tissue, and is therefore particularly beneficial for tumors close to vital organs or in treating children and young people. Both methods aim to exploit the sensitivity of cancer cells to DNA damage, leveraging the difference in repair capabilities between healthy and cancerous cells to selectively target tumors. The choice between photon and proton radiotherapy depends on tumor characteristics, field of treatment and the need to minimise damage to healthy tissue while effectively treating cancer.

When cells are exposed to ionizing radiation, be it through photons or protons, the energy imparted by the irradiation leads to the formation of damage within the cellular DNA (Bouwman and Jonkers 2012). This includes single-strand breaks (SSBs) and the more lethal double-strand breaks (DSBs). SSBs, although often efficiently repaired by the cell, can lead to mutations or, for instance via stalled replication forks, lead to DSBs. DSBs are particularly harmful because they can lead to cell death if not repaired correctly, or they can cause mutations and genomic translocations that may lead to further genomic instability. The cell attempts to repair this damage using various mechanisms, such as non-homologous end joining (NHEJ) and homologous recombination (HR) for DSBs (Ciccia and Elledge 2010). If the damage is irreparable, the cells may undergo apoptosis. However, often cells that have undergone significant genetic damage still attempt to divide. The resulting daughter cells are usually non-viable and die during or after mitosis due to the inability to properly segregate chromosomes, known as mitotic catastrophe (Ciccia and Elledge 2010).

2.2. Impact of cancer treatment on developing germ cells

2.2.1. The testis and male germ cell development

The testis is the primary male reproductive organ; its main functions are production of gametes (spermatozoa) via a process known as spermatogenesis, and production of the hormone testosterone. Structurally, the testis is composed of seminiferous tubules, the site of spermatogenesis, which are surrounded by supporting interstitial tissue. The chief cells of the testis are the germ cells, the Sertoli cells, the Leydig cells, and the peritubular myoid cells.

The evolution of the germ cells begins in fetal life, when the primordial germ cells (PGCs) migrate to the gonadal ridge. In the presence of the Sex determining Region on the Y chromosome (SRY) gene, there is expression of SOX9 in Sertoli cells and subsequent male sex determination; in this context, the PGCs develop into gonocytes (Hanley et al., 2000). Throughout fetal and neonatal life, gonocytes proliferate and asynchronously differentiate into pre-spermatogonia, such that the neonatal testis will contain both germ cell types (Mitchell et al., 2008; Wu et al., 2009). Throughout fetal and neonatal life there is concurrent development of the somatic cell population, with Sertoli cells

proliferating within the seminiferous cords, the precursor to seminiferous tubules (Sharpe et al., 2003); peritubular myoid cells developing to form the walls of the cords (Mayerhofer 2013); and fetal Leydig cells producing testosterone in utero, before regressing after birth, to be ultimately replaced by a new population of Leydig cells during puberty (Chen et al., 2009; Teerds and Huhtaniemi 2015), which will be responsible for testosterone production in adulthood.

During the first 3 months of post-natal life, there is a period known as mini-puberty, characterised by activation of the hypothalamic-pituitary-gonadal (HPG) axis and increased testosterone production (Forest et al., 1974; Svechnikov et al., 2010). During this time, the gonocytes migrate from the centre of the tubules to the basement membrane, differentiate into spermatogonia, including SSCs. In the period between infancy and onset of puberty, the spermatogonial population proliferates at a slower rate, with a subset differentiating into the type B spermatogonia which will ultimately enter meiosis and progress to mature spermatozoa (de Rooij 2001, Stukenborg et al., 2014). This process begins during puberty and continues throughout adulthood, supported by the somatic cells and governed by the HPG axis.

2.2.2. Effect of cancer treatment on differentiating germ cells

Chemo- or radiotherapy can have both immediate and long-term effects on male fertility. Spermatogenesis in adults involves transition from differentiating spermatogonia into a series of germ cell types up to mature spermatozoa that each respond differently to the cancer treatment.

Treatment can result in ejaculated spermatozoa with mutagenic damage, which will depend on the developmental stage at which they were exposed (Meistrich 2020). From studies mostly conducted in mice, but also some human, it has become clear that the rapidly dividing differentiating spermatogonia are more susceptible to most treatments than the later meiotic and haploid germ cell types (Oakberg 1955, Rowley et al., 1974; Meistrich et al., 1982). Spermatocytes are most sensitive to topoisomerase inhibitors; this is likely due to the fact that spermatocytes undergo meiotic recombination and topoisomerases play a role in this (Russell et al., 2000). Meanwhile spermatids and spermatozoa appear most sensitive to alkylating agents and radiation. Detailed lists of chemotherapeutic agents (including alkylating compounds) and irradiation, and their effect on different spermatogenic germ cell types, have been extensively reviewed (Howell and Shalet 2005; Meistrich 2013, 2020).

The majority of chemotherapeutic drugs are given as part of multi-agent therapy, therefore it can be difficult to ascertain the gonadotoxicity of each individual agent. Additionally, combining agents may have an additive or multiplicative impact on fertility. It is widely accepted that certain agents are more associated with gonadotoxicity, such as alkylating and platinum-based agents. The effects are related to cumulative dose, but can also be affected by combination therapy and host factors.

Importantly the kinetics of spermatogenesis and organization of the stages of the seminiferous epithelium remain unchanged in response to gonadotoxic stress (Edwards and Sirlin 1958). Hence, although carrying genomic damage caused by the treatment, directly damaged spermatocytes and spermatids may survive and contribute to spermatozoa in the semen. This is clinically important when considering the safety of cryopreservation of semen or conceiving children during, or immediately after, treatment with chemotherapy. Following gonadotoxic cancer treatment, there is a progressive loss of more mature differentiating germ cells with depletion up to the leptotene spermatocytes at 2 weeks after exposure and depletion of pachytene spermatocytes by 25 days after chemotherapy. Depending on toxicity of chemotherapy to later stage germ cells, 10–100-fold decline in sperm count may occur within 1–2 months, but azoospermia usually does not occur until after 2 months when the sperm would be derived from differentiating spermatogonia (Meistrich 2013, 2020). Therefore, it is generally recommended that patients wait (3 months or longer depending on the therapy) after a gonadotoxic treatment before conceiving children.

The testis is extremely sensitive to irradiation. A single dose as low as 2–3Gy can induce azoospermia, whereas permanent azoospermia may be anticipated at cumulative doses of >6Gy (Mitchell et al., 2017). TBI involving exposure to 10–13 Gy has been shown to cause azoospermia in 85% of men, with oligozoospermia reported in the remaining patients and is primarily due to direct damage to the testis (Anserini et al., 2002).

2.2.3. Effect of cancer treatment on spermatogonial stem cells

Whilst damage to the differentiating germ cells does not preclude recovery of spermatogenesis after treatment, survival of the SSC population is required. SSCs in the mouse are the spermatogonial cell population more resistant to severe DNA damage than differentiating spermatogonia, such as that caused by chemotherapy and ionizing radiation (van Beek et al., 1990; van der Meer et al., 1992a,b). For irradiation, this mostly affects dividing cells, which include the mitotically active spermatogonia (Oakberg 1955, Rowley et al., 1974; Meistrich et al., 1982). Spermatogonia are also prone to actively undergo apoptosis (Beumer et al., 1998; Hamer et al., 2003), which makes them very sensitive to radiotherapies. However, whilst the differentiating germ cells that were directly exposed to the treatment are no longer present in the testis after around 3 months, spermatozoa in the semen will be derived from SSCs that have been exposed to treatment. The reason for the relative insensitivity of SSCs to DNA damaging treatments may lie in the fact that, in contrast to their differentiating counterparts, they are not yet committed to follow stage-dependent cell division (de Rooij 2017). This allows SSCs to enter cell cycle arrest and repair the damage, thereby forming the potential basis for spermatogenic recovery after treatment. However, it is possible that surviving SSCs will carry mutations that escape repair; given that these progenitor cells give rise to future germ cells, this could result in sperm carrying these mutations, with potential implications for future fertility and offspring (Choy and Brannigan 2013, Seppanen et al., 2016).

2.2.4. Effect of chemotherapy on the developing testis during pre-pubertal life

Whilst spermatogenesis is not present in the pre-pubertal testis, SSCs are present; as a result, future fertility can be impacted by exposure to chemo- and radiotherapy. Several studies clearly show that the pre-pubertal testis may also be damaged by cytotoxic drugs. Nearly all patients receiving chemotherapy containing alkylating compounds before puberty had a mean tubular fertility index (TFI; percentage of seminiferous tubules containing identifiable spermatogonia) that was 50% or lower than that of age-matched controls, which is suggested to be independent of age of treatment (Lendon et al., 1978; Shalet 1980, Hensle et al., 1984; Averette et al., 1990; Wallace et al., 1991). Also numbers of spermatogonia per tubular cross section (S/T) in testes of pre-pubertal boys exposed to alkylating agents was significantly reduced compared with patients treated with non-alkylating agents (Poganitsch-Korhonen et al., 2017).

2.2.5. Potential for recovery of spermatogenesis after cancer treatment

Alkylating agents are often used in treatment of paediatric tumors and may cause prolonged or permanent azoospermia. The duration and permanence of the induced azoospermia depends on the dose of the cytotoxic agent and additive effects of different agents. Long-term impairment of spermatogenesis and decreased fertility is more likely after cumulative CEDs >4000 mg/m^2 (Green et al., 2014; Poganitsch-Korhonen et al., 2017). Platinum-based agents, such as cisplatin and carboplatin, are not currently included in the CED calculation but have been demonstrated in experimental and clinical studies to be damaging to the testis (Tharmalingam et al., 2020; Tian En et al., 2020). Some agents, including the anthracyclines (e.g. Adriamycin), microtubule inhibitors (e.g. vincristine), and select antimetabolites (e.g. cytarabine) do not produce prolonged azoospermia unless they are combined with more highly gonadotoxic agents (Meistrich 2013; Clark et al.,

2023).

Ultimately, the eventual recovery of sperm production depends on the survival of the SSCs, the regeneration of their numbers, and their ability to differentiate. In adult humans, as in rodents, following treatment with chemotherapy agents that do not result in loss of SSCs, there is usually a return of normal sperm count within 12 weeks after the cessation of chemotherapy. Many recover to normozoospermic levels, although some may have persistent oligozoospermia. For patients receiving a regimen including a low dose of cyclophosphamide, recovery to normozoospermia often begins at about 1 year, but can take 5 years to recover fully in up to 70% of patients. Certain treatment regimens, such as those involving high dose alkylating chemotherapy and/or direct testicular irradiation, are associated with a higher risk of permanent azoospermia. The effect is likely to be dose-dependent, as demonstrated by the strongly decreased recovery to normozoospermic levels in patients receiving higher doses of cyclophosphamide (>7.5 g/m^2) for Ewing's sarcoma (10%) compared to those receiving doses <7.5 g/m^2 (70%). Higher doses of cyclophosphamide having been demonstrated to increase risk of permanent azoospermia (Meistrich et al., 1992). Cumulative exposure is also likely to be important in determining likelihood of recovery from radiation-induced azoospermia; while a threshold dose of testicular irradiation has not been defined, irreversible damage is more likely after doses of $\geq$1.2 Gy (Howell and Shalet 2005), which is well below doses used therapeutically. However, because of the uncertainties involved, patients who do not wish to conceive should be advised to use appropriate contraception, even if they have previously received potentially very gonadotoxic therapy.

For cancer survivors who present with azoospermia after cytotoxic cancer therapy, it is possible that some sperm are being produced in the testis. Studies have shown that when the human testis contains fewer than 3–4 million sperm, these sperm may not survive epididymal transit to reach the ejaculate (Silber et al., 1997). Therefore surgical sperm retrieval from the testis using testicular sperm extraction (TESE) may be attempted. This has been reported to have been successful in 37% of patients that were azoospermic after chemotherapy (Hsiao et al., 2011).

2.3. Cancer treatment and the HPG-axis

2.3.1. The hypothalamic pituitary gonadal (HPG)-axis

The hypothalamic pituitary gonadal (HPG)-axis is the hormonal axis responsible for the regulation of the testis in terms of testosterone production and spermatogenesis. The axis involves the hypothalamus releasing gonadotropin-releasing hormone (GnRH), which prompts the pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates testosterone production from the Leydig cells in the testis, while FSH acts directly on the Sertoli cells to support spermatogenesis. The finely tuned balance of these hormones, involving a negative feedback loop from the testis to the hypothalamus and pituitary is critical for maintaining testosterone production and normal spermatogenesis throughout adulthood (Corradi et al., 2016). The axis is also active in fetal and early postnatal life, but then becomes quiescent throughout childhood until being reactivated at puberty and remaining active throughout adult life (Rohayem et al., 2024).

2.3.2. Impact of gonadotoxic testicular damage on HPG axis in adulthood

Direct gonadotoxic damage to the testis will result in impairment of spermatogenesis and, less frequently, testosterone production, which is largely due to the reduced sensitivity to cancer treatment of the Leydig cells in comparison to the proliferative germ cells in the testis (Mitchell et al., 2009). Nevertheless, direct damage of chemotherapy on the somatic environment results in a lack of negative feedback to the hypothalamus and pituitary and subsequent increase in gonadotropins, known as hypergonadotropic hypogonadism. Where Leydig cells are affected, testosterone levels may still remain in the normal range as a result of an increase in LH, so-called compensated Leydig cell failure (Cattoni et al., 2023). Chemotherapy primarily induces direct damage to testicular function, whilst irradiation can cause damage to either the testis or the hypothalamo-pituitary unit (Mitchell et al., 2009).

Alkylating agents have been shown to interfere with testicular somatic cell function and this is reflected in the response of the HPG axis to these treatments. Following alkylating agent exposure, the germinal epithelium becomes depleted and atrophic, with peritubular fibrosis occasionally noted, while Sertoli cells and Leydig cells appear histologically normal (Shalet 1980). However, in a study of 53 adult males (aged 16–45 years) with Hodgkin's disease, treatment with MOPP (nitrogen mustard, vinblastine, procarbazine and prednisone) regimen resulted in a higher percentage of patients with elevated FSH levels, high to normal LH concentrations in the presence of normal to subnormal testosterone concentrations and lower percentage of returning spermatogenesis, compared to treatment with ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) (Shalet 1980, Viviani et al., 1985; Averette et al., 1990). The raised FSH may be indicative of direct damage of treatment on Sertoli cells, although it may be more likely an indirect effect of germ cell loss, which subsequently impairs Sertoli cell function. Median luteinising hormone (LH) levels were at, or just above, the upper limit of normal, and median testosterone levels were normal. It is suggested that slightly elevated LH and slightly decreased testosterone indicate Leydig cell damage due to the cytotoxic combined therapy via a reduction in hypothalamus-pituitary negative feedback. Further analyses indicate that this mild Leydig cell insufficiency in some patients is associated with symptoms of hypogonadism such as decreased bone mineral density, reduced sexual activity and alterations in mood (Howell and Shalet 2002).

The testis is extremely sensitive to irradiation, and this may also be reflected in the response to damage of the HPG axis. A single dose as low as 2–3Gy can induce azoospermia, whereas permanent azoospermia may be anticipated at cumulative doses of >6Gy (Mitchell et al., 2017) Total body irradiation (TBI) involving exposure to 10–13 Gy has been shown to cause azoospermia in 85% of men, with oligozoospermia reported in the remaining patients. The effect on the HPG axis with raised gonadotropins indicates that these effects are mediated by direct damage to the testis (Anserini et al., 2002).

2.3.3. Impact of gonadotoxic testicular damage on HPG axis in childhood

While direct effects of cancer treatment on the testis in adult patients may be reflected in abnormalities of HPG axis resulting in changed FSH, LH and testosterone secretion (Shalet 1980; Howell and Shalet 2002), pre-pubertal testicular cell impairment will not be detected until puberty as the HPG axis is quiescent in childhood. It had been incorrectly assumed that the pre-pubertal testis would be more resistant to gonadotoxic agents than the pubertal or adult testis, because of the low cell proliferation rates, immaturity of Sertoli cells, lack of adult Leydig cells and relative quiescence of the HPG-axis (Averette et al., 1990; Meistrich 2009). However, the germ cells are sensitive to treatment in prepuberty, as indicated by an increase in FSH and azoospermia in adulthood. However, the progenitor Leydig cells appear to be relatively resistant to cancer treatment in childhood. In a study of male patients treated for Hodgkin's lymphoma with or without alkylating agents, data on recovery of fertility were available for 101 patients. Recovery to normal FSH levels occurred in 82% of patients treated without alkylating chemotherapy, compared to 30% of those treated with alkylating agents; additionally, time to recovery was statistically longer in those receiving alkylating agents (van der Kaaij et al., 2007). Even when subsequent azoospermia is irreversible, the majority of boys treated with combined chemotherapy, such as MOPP, progress through puberty normally and do not require testosterone replacement indicating intact Leydig cell function after treatment (Averette et al., 1990).

2.3.4. Impact of cancer treatment on the hypothalamus and pituitary

In addition to direct damage to the testis, cancer treatments can also impact testosterone production and spermatogenesis in adulthood

through direct cancer treatment effects on the hypothalamus or pituitary gland, ultimately leading to reduced LH, and FSH secretion. Reduced levels of these hormones results in decreased stimulation of the testes leading to hypogonadotropic hypogonadism with lowered testosterone levels and impaired spermatogenic function. Despite the relative quiescence of the axis during childhood, the hypothalamus and pituitary are still sensitive to the effects of cancer treatment, primarily irradiation and this has implications for subsequent puberty, testosterone production in adulthood and future fertility. This has been shown in a study of 45 children treated with cranial radiation in whom severe gonadotropin deficiency occurred in 11% of cases (Rappaport et al., 1982).

Simultaneous damage to the brain and testis, will have competing effects on gonadotrophin production with hypothalamus-pituitary damage causing reduced gonadotropins, whilst testicular damage resulting in reduced testosterone will lead to increased gonadotropins. The net effect may be gonadotropin levels within the normal adult range, which may provide false reassurance that testicular function may be preserved. However, in such situations reduced testicular volume, low testosterone or impaired sperm production may indicate the presence of hypogonadism.

The level of the HPG axis where the damage occurs is also important in terms of rescue of testicular function. For example, if the damage occurs primarily at the level of the hypothalamus or pituitary e.g. with high-dose cranial irradiation, then testosterone production and spermatogenesis may be induced by stimulation with exogenous gonadotropins. This has been shown in men with genetic forms of hypogonadotropic hypogonadism (Prior et al., 2018); whereas if the damage primarily occurs within the testis, leading to hypergonadotropic hypogonadism, then gonadotropin treatment will not be effective as a treatment option.

3. The effect of the evolution of chemotherapeutic regimens on fertility

3.1. Adaptation of chemotherapy to prevent late adverse effects other than infertility

Since their introduction, cancer treatments have been known to have detrimental off-target effects, and development of drugs and treatment protocols has focused on maintaining or improving efficacy of anti-cancer treatment whilst mitigating associated toxicities (Fig. 1). Initial treatment modifications were largely focused on minimising acute, severe, and often life-threatening toxicities, such as myelosuppression leading to sepsis. Whilst these remain important, they can be effectively managed with appropriate and timely supportive care. As survival rates have improved, there has been an increasing focus on survivorship and the reduction of late effects, such as cardiotoxicity, ototoxicity, growth impairment and gonadotoxicity. It is possible that treatment modifications which aim to reduce the general toxicity or alleviate specific acute or long-term side effects of treatment may also have the serendipitous effect of reducing the negative impact on fertility and reproductive function.

However, it cannot be assumed that a reduction in one or more specific treatment-related toxicity will result in a reduction in gonadotoxicity. Cisplatin is a platinum-based alkylating agent which is commonly employed in multiple paediatric oncology treatment regimens; it is associated with risk of irreversible sensorineural hearing loss and with nephrotoxicity, which in some cases leads to end-stage renal failure (Coradini et al., 2007; Plumb et al., 2018). In 1989, the FDA approved the use of carboplatin as an alternative platinum-based agent; carboplatin is associated with a lower risk of ototoxicity and nephrotoxicity than cisplatin (Oun et al., 2018). However, carboplatin has been demonstrated in *in vitro* studies to cause similar gonadotoxicity as cisplatin, in terms of reduced spermatogonial number in the pre-pubertal human testis (Tharmalingam et al., 2020).

Whether this translates into long-term clinical outcomes is not known. There are limited data to directly compare the effects of treatment in childhood with cisplatin and carboplatin on subsequent fertility (Tian En et al., 2020). However, these agents are commonly employed to treat testicular tumors in male patients of reproductive age. In a study of young male patients with testicular germ cell tumors, sperm parameters, including aneuploidy and DNA integrity and fragmentation in patients treated with bleomycin, etoposide, and cisplatin (BEP; n = 100) were compared to patients treated with carboplatin (n = 54) or surveillance-only (n = 58). One year after treatment, the patients treated with BEP had a significant reduction in sperm count and concentration, and significantly higher rates of sperm aneuploidy and DNA fragmentation (both single- and double-stranded breaks), compared to patients treated with carboplatin and surveillance-only patients. There was a slight improvement in sperm count two years post-treatment, with no change in aneuploidy or DNA damage. Of note, there was no evidence of aneuploidy or DNA compaction or fragmentation in patients treated with carboplatin-only at one- or two-years post-treatment (Ghezzi et al., 2016). This suggests that carboplatin may be a less gonadotoxic treatment option, although it is important to consider that the study discussed compared single-agent carboplatin to multi-agent therapy. A similar study examining sperm counts in 119 patients with stage 1 testicular cancer, aged 18–50 years, treated with either BEP, carboplatin, or radiotherapy found no significant difference in total sperm number or sperm concentration between treatment groups (Weibring et al., 2019). While there are limited clinical data on outcomes in paediatric patients, the AGCT1531 trial is currently comparing treatment with carboplatin and cisplatin for paediatric and adult patients with germ cell tumors, comparing both efficacy and toxicity between these agents (Clinicaltrialregister, 2020).

Another example of an important change in treatment which aims to minimise toxicity is the evolution in conditioning regimens for haematopoietic stem cell transplantation (HSCT), which have undergone significant changes over recent decades. HSCT has been employed since the 1950s, for both benign and malignant indications; however, in the early decades the treatment was ultimately unsuccessful, with patients largely succumbing to graft failure or graft versus host disease (GVHD) (Bortin 1970). Early conditioning regimens involved exposing patients to a single-dose TBI; the introduction of fractionated radiation (whereby the same cumulative dose is administered in staggered fractions) and chemotherapy conditioning, through such agents as cyclophosphamide and busulfan, led to improved outcomes (Santos et al., 1983). However, these conditioning regimens continued to be associated with significant acute and long-term toxic effects, leading to significant morbidity and mortality. The treatment modalities utilised – TBI and high dose alkylating chemotherapy – are amongst the most gonadotoxic treatment regimens, and infertility is a common late effect of HSCT (Chow et al., 2016; Mulder et al., 2021).

Recent decades have seen the introduction of reduced-intensity conditioning (RIC) regimens, using non-myeloablative chemotherapy and no or lower doses of TBI, in part to minimise toxicity. However, there remains limited data on long-term fertility outcomes of male patients treated with RIC. Indeed, a retrospective cross-sectional study examining male patients treated in childhood/young adulthood with RIC versus conventional myeloablative regimens found no significant difference in the prevalence of gonadal failure or azoospermia between the treatment groups (Bender et al., 2023). Given that RIC is a relatively newer treatment modality, follow-up duration is limited in current studies and further long-term outcome data is required.

3.2. Adaptation of chemotherapy to prevent late adverse effects in fertility

Given the increased awareness of the impact of cancer treatment on fertility and the importance that this holds for patients and their families, it can be argued that there is a need to modify treatment protocols with the specific aim of reducing gonadotoxicity. One example of this is changing treatment of Hodgkin's lymphoma. In an analysis combining

data from four randomised controlled trials, spanning 1982–2004, fertility was assessed in 355 adult male patients. In this cohort, there was evidence of impaired spermatogenesis (elevated FSH) in 60% of patients treated with alkylating chemotherapy, compared to 8% treated with non-alkylating chemotherapy and 3% treated with radiation. Patients treated with alkylating chemotherapy were less likely to have recovery of spermatogenesis than those treated with non-alkylating chemotherapy (van der Kaaij et al., 2007).

In 2022, the results of a large multicentre randomised trial (EURONET-PHL-C1) for treatment of Hodgkin's disease under the age of 18 demonstrated that replacing the alkylating agent procarbazine with an alternative alkylating agent, dacarbazine, resulted in a significant reduction in gonadotoxicity. FSH was elevated in 66% of patients treated with COPP (cyclophosphamide, vincristine, prednisolone, and procarbazine), compared with 9% of patients treated with COPDAC (cyclophosphamide, vincristine, prednisolone, dacarbazine). Additionally, in this cohort, azoospermia was seen in 83% of patients treated with COPP and none of the patients treated with COPDAC (Mauz-Korholz et al., 2022). In order to assess the safety of removing procarbazine from the treatment regimen, a randomised controlled trial is ongoing. It is notable that this treatment is being changed with the specific aim of reducing gonadotoxicity and improving fertility outcomes for patients, whilst maintaining efficacy; this is an important step forward in improving fertility outcomes after cancer treatment. It has been recommended that assessment of reproductive function should be incorporated into both routine follow-up and clinical trials; this would facilitate ongoing modification of treatment regimens to protect fertility (Anderson et al., 2021).

Balancing efficacy with prevention of treatment-associated toxicity will be particularly important when considering the impact of targeted and novel therapies on fertility. For example, recent decades have seen significant changes in the treatment of ALL, associated with an increase in overall survival rates to over 90% (Winter et al., 2018; Pedrosa et al., 2020). This is partly attributable to therapeutic modifications which have made to reduce treatment-associated toxicities. An important change in the standard of care has been the approach to central nervous system (CNS) disease. Historically, prophylactic cranial irradiation was given as part of standard ALL treatment, and this was associated with significant long-term morbidities, including secondary malignancy, cognitive difficulties, and endocrine dysfunction (Conklin et al., 2012). This had implications for fertility due to the effect on the HPG axis; in a study of 213 males treated for childhood ALL, men who had been treated before the age of 10 with high dose (24Gy) cranial radiotherapy had reduced fertility compared with controls (Byrne et al., 2004). However, optimisation and systemic and intrathecal chemotherapy have allowed for the removal of prophylactic cranial irradiation from ALL treatment protocols, thereby eliminating concern about hypothalamic-pituitary-related infertility in this patient cohort (Pui et al., 2009).

More recent changes in treatment of ALL can be attributed to advances in cytogenetic and molecular profiling, which have contributed to improved understanding of disease pathology with consequent development of targeted treatments and immunotherapy. For example, the tyrosine kinase inhibitor Imatinib was found to improve survival in patients with Philadelphia chromosome-positive ALL, without increased risk of acute toxicities (Schultz et al., 2009). However, relatively little is known about the effect of treatment with Imatinib, particularly in childhood, on subsequent fertility. A study of 150 patients treated in adulthood with Imatinib or other tyrosine kinase inhibitors for chronic myeloid leukaemia (CML) found no reduction in fertility (Abu-Tineh et al., 2023). However, a further study of 50 adult male CML patients treated with imatinib found that treated patients had reduced sperm count and density compared to controls (Chang et al., 2017). It will be particularly important to gather longer-term follow-up data on reproductive outcomes in young male patients to gain a more comprehensive understanding of the impact on fertility.

3.3. Adaptation in radiotherapy to prevent late adverse effects in fertility

As well as changes in drug treatment, recent decades have seen significant change in the approach to radiotherapy, with increasing use of proton treatment. Endocrinopathies, including central hypogonadism, have been a significant long-term morbidity of cranial radiotherapy for CNS tumors. In a study assessing endocrine outcomes of 77 children treated for medulloblastoma with either proton or photon radiation, proton radiotherapy was associated with a reduced risk of sex hormone deficiency compared to photon radiation (Eaton et al., 2016). There remains relatively little data on the fertility outcomes in male patients treated with proton therapy for CNS disease.

3.4. Looking forward: the impact of cancer treatment on male fertility in the future

As previously discussed, recent decades have seen the emergence and increasing use of immunotherapy and targeted therapies in cancer treatment protocols. For many diseases, these therapies have significantly improved survival rates and become standard treatments, such as the use of the chimeric CD20 antibody Rituximab for non-Hodgkin's lymphoma (Grillo-Lopez et al., 2000; Dotan et al., 2010). Of note, Rituximab is also used in the treatment of non-malignant conditions, such as rheumatic diseases. However, there remains limited data on the effect of Rituximab on male fertility. Indeed, a recent systematic review of the effect of immunosuppressive drugs on male reproductive outcomes (including sexual function, reproductive hormones, fertility and pregnancy/offspring) found no eligible studies focusing on the effects of Rituximab (Perez-Garcia et al., 2020). As the use of such immunotherapeutic agents continues to expand, it is important to actively investigate the effects of these drugs on fertility. It is also important to consider that the administration of immunotherapy often differs from that of standard chemotherapy, with patients being on treatment for significantly longer durations; this makes assessment of toxicity challenging and also poses the question of how to advise patients about when it is safe to attempt to conceive.

Additionally, novel anti-cancer treatment modalities continue to be developed and employed through clinical trials, such as the use of blinatumomab and CAR-T cell therapy in ALL. For these agents, very little is known about the impact on fertility. While it can be speculated that more targeted treatments may have fewer off-target effects and therefore a lesser detrimental impact on fertility, that cannot be assumed. The ongoing introduction of novel treatments should be seen as an opportunity to improve our understanding of the effects of cancer treatment on fertility, through the incorporation of reproductive assessment into clinical follow-up and trial outcomes (Ligon et al., 2022).

4. Fertility preservation and protection: a balancing act between expected gonadotoxicity and patient desires

4.1. Fertility preservation

According to the World Health Organization (WHO), currently 8% of cancer cases in men affect those under the age of 45 globally, of which 11% are (pre)pubertal (World Health Organization 2022) These patients may have an active child wish or a desire to start a family in the future. From previous research it is known that (childhood) cancer survivors in general wish to have biological children (Schmidt et al., 2016). Being infertile can cause low self-esteem, marital problems and psychological suffering (Langeveld et al., 2004; Zebrack et al., 2004; Crawshaw and Sloper 2010), which can affect the desire or ability to have a child. Fertility preservation options are available to safeguard the opportunity of biologically-own children for them, although these options are often lacking in developing countries (Ataman et al., 2022). For young adult cancer patients, proper counseling for fertility preservation is recommended for any dose of alkylating agents, testicular or cranial

radiotherapy, HSCT or when an orchiectomy is indicated (Mulder et al., 2021).

For post-pubertal patients, the cryopreservation of sperm from the ejaculate prior to treatment may allow for these sperm samples to be used for medically assisted reproduction after cure from the disease. Whilst not all patients use their sample, it is still considered a cost-effective strategy for fertility preservation for post-pubertal males (Gilbert et al., 2018). It has been estimated that 15% of all men that survive their cancer come back to use their sample for reproductive use, while a similar percentage choose to discard the sample, supposedly because there is no longer a desire for children or fertility has returned naturally (Ferrari et al., 2016). The use of cryopreserved sperm to achieve parenthood is successful in approximately 50% of cases. Given that sperm cryopreservation is non-invasive, relatively inexpensive, and the chances of siring a successful pregnancy are good, it should be offered to all male cancer patients facing gonadotoxic treatment. For patients who are unable to produce a semen sample, electro-ejaculation or penile vibratory stimulation, both performed under a general anaesthetic are alternative strategies to obtain sperm from the ejaculate (Gat et al., 2014). When no sperm is found in the ejaculate, surgical approaches including onco-TESE may be considered (Fig. 1).

Because of their young age, sperm cryopreservation is not an option for pre-pubertal boys. For them a testicular biopsy to preserve SSCs, may provide a solution. The current data indicates that the cryopreservation of a testicular biopsy is feasible and safe, with a low complication rate post-biopsy (Ginsberg et al., 2010; Uijldert et al., 2017; Ming et al., 2018). Several therapies are in preclinical development to use this biopsy for fertility treatment, all which revolve around the differentiation of the SSCs present in the biopsy, either *in vivo* or *in vitro* (reviewed by (Sanou et al., 2022)). For both spermatogonial stem cell transplantation and testicular tissue grafting, primate data of efficacy exists (Fig. 1), and ethical approval has been provided for first clinical trials, while human *in vitro* spermatogenesis is still in its infancy. Even though exciting, at this moment in time, we are still uncertain whether these methods will be proven effective in these clinical trials. In addition, the number of spermatogonia was found to be lower in boys with some types of cancer even before chemotherapy (Masliukaite et al., 2023).

Because of the level of uncertainty regarding the potential future use, and the high costs associated with these treatments, it is important that only those patients that are at high risk of becoming infertile after cancer treatment are offered testicular tissue cryopreservation. The current recommendations for male pre-pubertal and adolescent cancer patients to be eligible for fertility preservation via a testicular biopsy are those that are treated with high-dose alkylating agents or equivalent gonadotoxic chemotherapy (i.e., CED $\geq$4000 mg/m^2), testicular radiotherapy or myeloablative HSCT (Mulder et al., 2021). A proper prediction of the expected damage to the testis is vital when deciding to offer fertility preservation. The ongoing evolution in cancer treatments, with unknown fertility outcomes, creates an extra level of complexity to the practical usage of these guidelines.

4.2. Research to predict or prevent testicular damage

Gonadotoxicity is traditionally tested in small rodents, however due to the phylogenetic distance between rodents and human, testis development diverges between species (Cunha et al., 2023). Consequently, results obtained in rodent studies may not be fully translatable to the human situation. In addition, testing the full spectrum of newly developed regimens, would require a vast number of animals.

To predict the potential gonadotoxicity of a (novel) anti-cancer drug or regimen in humans, testicular organoids may provide a solution (Pendergraft et al., 2017; Nishimura and Takebe 2024). Organoids generally possess micro-anatomical structures resembling the original organ, and offer the potential to study cell-cell communication and interactions (Sakib et al., 2020; Nishimura and Takebe 2024). Technically, these organoids can be described as reconstituted testis displaying functional similarities to human testes, such as a retinoic acid response and a blood-testis barrier (Alves-Lopes et al., 2017; Sakib et al., 2019) A multi-organ-on-a-chip format, combining both testis and liver, might provide a more comprehensive platform to include the impact of drug activation or metabolism of the agents in the model (Baert et al., 2020). Although still in development, testicular organoids could offer a high-throughput and potentially personalized approach to study spermatogenesis and conduct gonadotoxicity assays. This would aid prediction of the infertility risk after treatment, and therefore, the need for fertility preservation prior to treatment.

Both organoids and organotypic testicular cultures are useful in development of cytoprotective agents or protecting testis using hormone suppression. This could serve as a potent strategy to minimise reproductive harm during cancer treatment (Allen et al., 2018). These cytoprotective agents are specifically designed to protect the immature germ cells. Current work has focused on protection from damage caused by chemotherapies such as cisplatin (Matilionyte et al., 2023) and doxorubicin (Ujah et al., 2021). In the future effective cytoprotection may make fertility preservation obsolete.

5. Concluding remarks

In conclusion, even though oncological treatment has resulted in significant advances in the care of cancer patients of all ages, gonadotoxicity remains an issue. Whilst there have been successes in reducing general toxicity of treatments, this does not necessarily result in reduced gonadotoxicity. Therefore, fertility preservation programs are essential, along with fundamental research into how cancer therapies affect the testes and how this may be alleviated. Through the combined action of clinical oncologists and reproductive biologists, within the context of wider clinical and research teams, we continue to strive towards a future where cancer patients can not only overcome their disease but also fulfill their dreams of starting a family.

Conflict of interest

The authors report no financial or personal conflict of interest relevant to this work.

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