

# Male Fertility-Implications of Anticancer Treatment and Strategies to Mitigate Gonadotoxicity

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**Abstract:** With the advent of the modern cancer treatment, survival rates have improved substantially raising new concerns towards quality of life issues such as future fertility and offspring welfare. Cancer researchers are expanding their focus beyond survival and recurrence rates to include maximization of fertility potential for young cancer patients.

Despite promising cure rates with chemotherapy, studies have shown it to act as a double edge sword by adversely affecting male fertility. Chemotherapeutic agents act by hindering rapidly proliferating cells, hence exerting their gonadotoxic effect. The extent of damage to germ cells and eventual fecundity depends on the class of chemotherapeutic agent, dosage, spermatogenetic stage targeted as well as the original pretreatment fertility potential of the patient.

In this review, we provide a contemporary overview of the effects of anticancer agents on male fertility. Gonadotoxicity caused by these agents will be analyzed followed by the contemporary measures to preserve future fertility. Both established and potential strategies of fertility preservation will be discussed with emphasis on cryopreservation and its efficacy in conjunction with assisted reproductive technologies in addition to the current recommendations for this preservation modality. Finally, contemporary research on the welfare of offspring of cancer survivors will be reviewed.

## INTRODUCTION

National cancer records continue to demonstrate a significant annual increase in cancer incidence among the young American population [1]. The National Cancer Institute has estimated the number of newly diagnosed cancer patients in 2009 to reach 8400 for testicular cancer, 4640 for Hodgkin's disease (HD), 3350 for acute lymphocytic leukemia and 1430 for bone and soft tissue sarcoma [2]. Moreover males aged 15 to 45 years old show the highest incidence for these particular cancer types with testicular cancer reaching 84% of total cancers [3]. Fortunately the incline in cancer incidence has been paralleled by substantial improvements in cancer treatment modalities leading to similar increases in survival rates among many types of cancer. This allowed researchers to expand their focus of cancer treatment beyond primary issues of survival and recurrence rates towards the long-term quality of life of cancer survivors. Future fertility is an area of paramount concern to young cancer survivors. Over half of all seminoma patients rate future fecundity as an important issue even before cancer treatment [4]. In patients who had not caused a pregnancy previously, this concern was raised to 76% of seminoma patients [5].

Chemotherapy stands as a crucial arm in the battle against cancer either as solo treatment or in conjunction with surgery and radiotherapy. Chemotherapeutic agents are utilized in over 3000 reproductive-age men annually in addition to other cytotoxic lines of cancer therapy [6]. Knowing that chemotherapeutic agents target rapidly proliferating cancer cells, renders its gonadotoxic effect hardly surprising. As with radiotherapy, the type and dosage of the chemotherapeutic agent used, pretreatment semen quality and the developmental stage of the germ cells are all factors that determine the degree of gonadotoxicity caused by different chemotherapeutic combinations [7].

In this review, we provide a current and focused overview of the current literature on the gonadotoxic effects of contemporary anti-cancer agents used for the treatment of the two most common malignancies affecting the reproductive-age male population, namely Hodgkin's disease (HD) and testicular cancer. Our analysis extends beyond the adverse effect on the basic semen parameters to the changes in the genomic integrity of the sperm. In addition to

discussing the current strategies to maximize the fertility potential in patients receiving chemotherapy such as drug regimen modification and sperm cryopreservation, promising research will be reviewed in the areas of hormonal manipulation to minimize gonadotoxicity of cytotoxic agents, pre-pubertal testicular tissue preservation and cytoprotective adjuvants. We conclude with recent data on the health implications to offspring of cancer survivors who have a prior history of receiving anti-cancer drugs.

## GONADOTOXIC EFFECTS OF CHEMOTHERAPY

### Spermatogenetic Stage-Specific Cytotoxicity of Anti-Cancer Agents

Understanding the normal physiology of the different stages of spermatogenesis helps to infer and understand the extent of reproductive toxicity caused by anti-cancer agents. Early studies analyzed spermatogenetic cell toxicity by histologically examining testicular tissue after exposure to anti-neoplastic agents. The primary spermatocytic stage appeared to be the common stage relatively immune to chemotherapeutic gonadotoxicity while rapidly dividing A<sub>1</sub> spermatogonia represent the most sensitive target for cytotoxic agents [8]. Spermatogonia are classified into three types in order of development; stem cells, proliferative and differentiating spermatogonia [9]. Around 4-6 weeks after chemotherapy, fertility potential declines to its lowest levels, suggesting that differentiating spermatogonia are most vulnerable to chemotherapeutic gonadotoxicity [10]. Stem cells were found to be more resistant to chemotherapeutic toxicity than differentiating spermatogonia which may be attributed to their lower rate of proliferation [8]. The severity and duration of fertility impairment following chemotherapy correlated to both the number and stage of the germ cells damaged [8, 11]. Accordingly, each anti-cancer agent would have its unique sperm recovery rate based on its mechanism of action. In addition to the direct destruction of germ cells after crossing the blood-testis barrier, many chemotherapeutic drugs induce hyalinization and fibrosis of testicular interstitial tissue further reducing the number of functional germ cells [7].

### Anti-Cancer Agent Effects on Semen Quality and Fertility Potential (Table 1)

#### Single Agent Effects

A recent study documented that 6 months of chemotherapy caused severe oligospermia in 67 % and 60 % of treated testicular

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Table 1. Anti-Cancer Agent Effects on Semen Quality and Fertility Potential

Group Type	Subgroup	Anti-Cancer Agent	Effects on Semen Quality and Fertility Potential	Ref.
Alkylating agents	<u>Mustards</u>	Cyclophosphamide	<ul style="list-style-type: none"> <li>• Azoospermia</li> <li>• Permanent sterility</li> <li>• Decrease in testicular weight, and epididymal sperm count and motility</li> </ul>	[9,15] [16] [17]
		Cytarabine	• Azoospermia and severe oligospermia	[18]
		Ifosfamide	• Azoospermia and severe oligospermia	[18]
		Chlorambucil	• Azoospermia and severe oligospermia	[18]
	<u>Organoplatinum compounds</u>	Platinol (cis-platinum)	<ul style="list-style-type: none"> <li>• Irreversible impairment of spermatogenesis</li> <li>• Azoospermia and severe oligospermia</li> <li>• FSH and LH elevation</li> </ul>	[19, 20] [21, 22] [21]
	<u>Other alkylating agents</u>	Busulfan	• Failure of sperm regeneration	[23]
		Procarbazine	• Azoospermia and severe oligospermia	[9, 18]
		ThioTEPA	• Death of large numbers of germ stem cells	[24]
		Methyl methanesulphonate (MMS)	• Increase in morphologically abnormal sperm	[25]
		Dacarbazine	<ul style="list-style-type: none"> <li>• Severe oligospermia, asthenospermia and teratozoospermia</li> <li>• Reduction of intratesticular testosterone levels</li> </ul>	[26]
	<u>Agent combinations</u>	Cyclophosphamide and busulfan	• Failure of sperm regeneration	[27]
		CHOP	<ul style="list-style-type: none"> <li>• Failure of sperm regeneration</li> <li>• Decrease in seminiferous tubule diameters, reproductive organ weights and spermatozoal content of the testes and epididymides</li> </ul>	[28] [29]
		BEACOPP	<ul style="list-style-type: none"> <li>• Azoospermia and dysspermia</li> <li>• FSH elevation</li> </ul>	[30]
		MOPP	<ul style="list-style-type: none"> <li>• Permanent germ cell depletion</li> <li>• Azoospermia</li> </ul>	[8, 28] [31]
		MVPP	• FSH elevation	[32]
		COPP/ ABV	• Azoospermia	[33]
		BEP	<ul style="list-style-type: none"> <li>• Azoospermia</li> <li>• Reduced ejaculatory volume</li> </ul>	[28, 34] [35]
		ChIVPP	• Damage to seminiferous epithelium and FSH elevation	[36]
		BEAM	• Persistent azoospermia	[37]
	Non-Alkylating agents	<u>Topoisomerase II inhibitors</u>	Adriamycin	<ul style="list-style-type: none"> <li>• Azoospermia</li> <li>• Decrease in sperm count, motility, viability and normal morphology</li> <li>• Distorted testicular histology</li> <li>• Severe degenerative alterations in spermatogenic cells and seminiferous tubules with increased asthenospermia and teratozoospermia</li> </ul>
<u>Aneuploidy inducers</u>		Vinblastine	• Azoospermia and severe oligospermia	[18]
		Vincristine	<ul style="list-style-type: none"> <li>• Azoospermia</li> <li>• Deterioration in testicular weights, sperm counts and morphology</li> </ul>	[9] [40]
<u>Antimetabolites</u>		6-Mercaptopurine	• Azoospermia	[9]
		Fludarabine	• Reduced testicular volume, oligospermia, elevated FSH and LH and reduced testosterone levels	[41]
		Cytarabine	• Azoospermia and severe oligospermia	[18]
<u>Agent combinations</u>		ABVD	• Post-treatment sperm recovery rate of 90%	[28, 42]
		NOVP	• Azoospermia and severe oligospermia	[43]

cancer and HD patients respectively and was associated with a significant increase in abnormal sperm morphology. Nonetheless, sperm morphology proved to be the parameter least affected by anti-cancer drugs with restoration of sperm forms to baseline within 6 months of treatment cessation [12]. A critical analysis of single agent gonadotoxicity is difficult because most treatments incorporate multi-drug regimens. Generally, the side effects of anti-cancer agents depend on the cumulative result of the exact drug combination used according to the doses and frequency of administration. Pretreatment gonadal function is also an important determining factor of the extent of gonadal insult.

#### **Alkylating Agents**

Alkylating agents, particularly cyclophosphamide (CY) and procarbazine have the highest gonadotoxicity among the various cytotoxic agents [13]. Higher rates of spermatogenic recovery occur with intermittent compared to continuous use [14]. CY or procarbazine resulted in a 68% rate of azoospermia within a 20 year post-treatment follow up period [9].

#### **Mustards**

Single agent treatment with CY caused gonadal dysfunction in over 80 % of post-pubertal patients receiving doses exceeding 300 mg/kg [44]. A cumulative dose of 7.5 g/m<sup>2</sup> CY or above in CY-ADIC (cyclophosphamide, doxorubicin, and dacarbazine), or CY-VADIC (vincristine added to CYADIC) for Ewing and soft tissue sarcomas caused persistent sterility for 5 years after treatment discontinuation [16]. However, men treated with doses below 7.5 g/m<sup>2</sup> remained normospermic [15]. All patients treated before puberty had an abnormal semen analysis concurring with prior studies that disaffirmed the notion that prepubertal testes were immune to the gonadotoxicity of cytotoxic agents [15, 18, 31, 36, 45, 46]. Oh *et al.* documented significant decreases in testicular weight, epididymal sperm count and motility in rats exposed to CY [17]. Other mustard-class agents have shown similar suppression of spermatogenesis. In men treated with ifosfamide for Ewing's sarcoma, 60% were rendered azoospermic or oligospermic as well as the majority of those treated with cytarabine. Chlorambucil induced azoospermia in almost all treated subjects [18].

#### **Organoplatinum Compounds**

Platinol (cis-platinum) is a clear cause of irreversible impairment of spermatogenesis noted with cumulative dose exceeding 400-600 mg/m<sup>2</sup> [19,20]. Platinum-based regimens may also cause temporary azoospermia with spermatogenic recovery starting between the 2<sup>nd</sup> and the 4<sup>th</sup> year after cessation of therapy [21]. Severe oligospermia and azoospermia occurred in 19%, 47% and 100% treated with cumulative doses of 300 mg/m<sup>2</sup>, 600 mg/m<sup>2</sup>, and exceeding 600 mg/m<sup>2</sup> respectively [22]. Although Leydig cells show higher resistance to chemotherapy and radiotherapy damage owing to their slower rate of turnover, 45% of patients treated with a cumulative cis-platinum dose exceeding 600 mg/m<sup>2</sup> showed Leydig cell dysfunction compared to 27% of patients treated with lower doses [21]. Persistent azoospermia was recorded in 37% after 20 years post-cis-platinum treatment.

#### **Other Alkylating Agents**

Busulfan caused an additional 45% failure in spermatogenesis when used in combination with CY compared to solo CY treatment [23]. Azoospermia and severe oligospermia was evident in all patients treated with procarbazine for HD [18]. A study on the harmful effects of a variety of chemotherapeutic drugs on mice showed triethylenethiophosphoramide (thio-TEPA) unique for inducing a large degree of germ stem cell toxicity. Methyl methanesulphonate (MMS) treatment was associated with the development of morphologically abnormal sperm [25]. Dacarbazine resulted in severe oligospermia, asthenospermia and teratozoospermia in addition to reduced intratesticular testosterone levels [26].

#### **Non-Alkylating Agents**

Depending on the regimen and dose, non-alkylating agents may also exhibit profound gonadotoxicity. Treatment with non-alkylating agents, such as adriamycin (ADR), vincristine, methotrexate and 6-mercaptopurine, rendered 16% of men azoospermic for up to 11 years after treatment was stopped [9].

#### **Topoisomerase II Inhibitors**

Rats treated with ADR for 10 weeks displayed significant decreases in sperm count, motility, viability and normal morphology, in addition to distorted testicular histology [38]. ADR also caused severe degenerative alterations in spermatogenic cells and seminiferous tubules with increased asthenospermia and teratozoospermia [39].

#### **Aneuploidy Inducers**

Vinca alkaloids not only arrest spermatogenesis but also hinder sperm motility [47]. Azoospermia and severe oligospermia were apparent in all patients treated with vinblastine for HD [18]. Vincristine treatment caused reduction in testicular weights, sperm counts and morphology [40].

#### **Antimetabolites**

In a study of gonadotoxic effects of 14 chemotherapeutic drugs on mice, Meistrich noted that the least toxicity was after prednisone or 6-mercaptopurine treatment reaching down to nil in some cases [24]. The majority of patients treated with cytarabine suffered azoospermia or oligospermia after treatment [18]. Fludarabine resulted in reduced testicular volume, oligospermia, elevated follicle stimulating hormone (FSH) and luteinizing hormone (LH) and reduced testosterone levels within a month of treatment [41].

#### **Combination Treatment Effects**

##### **Alkylating Agent Combinations**

#### **Cyclophosphamide Based**

Spermatogenic recovery after CY and busulfan preparation before bone marrow transplantation occurred in less than 20% of treated cases [27]. CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy for non-Hodgkin lymphoma (NHL) was associated with a 67% recovery rate of spermatogenesis after 5 years [28]. In a rat model, CHOP administration induced declines in reproductive organ weights, spermatozoal content of the testes and epididymides as well as a 20% decrease in seminiferous tubule diameters [48]. Newer treatment regimens such as BEA-COPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) for advanced stage HD produce a high overall tumor response rate although almost 90% of patients develop azoospermia. Although testosterone and LH levels were less affected than with other HD regimens, FSH levels were abnormal in 93% of the subjects [30].

#### **Procarbazine Based**

Limited treatment cycles of MOPP (mustine, vincristine, procarbazine and prednisolone) were standard therapy for HD until the early 1990s. Together with other procarbazine regimens, these treatments induced permanent germ cell depletion and Sertoli-cell only testicular histology in the vast majority of men [28]. Six cycles of MOPP were suggested sufficient to destroy all testicular stem cells in man [8]. MOPP-induced azoospermia and oligospermia persisted in 63% and 32% of the patients respectively [31]. This gave rise to other combinations such as MVPP (mustine, vinblastine, procarbazine and prednisolone) that gave the same curative results with lower toxicity, but still containing the alkylating agent mustine. Howell and his group observed that MVPP caused a significant rise in FSH levels in over 90% of the patients denoting testicular dysfunction caused by these agents [32]. COPP (cyclophosphamide, vincristine, procarbazine and prednisolone)/ABV (doxorubicin, bleomycin, vinblastin) hybrid regimens retained high

levels of gonadotoxicity underscoring procarbazine as the main cause of gonadal damage [33].

#### Platinum Based

In a large series of testicular cancer patients treated with platinum-based regimens such as bleomycin, etoposide and cisplatin (BEP) for 3 years, patients were noted to have a 30% decline in their fertility potential compared to pre-treatment [49]. Spermatogenic recovery required at least two years after cessation of BEP therapy, ultimately recovering in 80% of the patients within 8 years [28, 34]. A large series of germ cell tumor patients who received POMB/ACE therapy (cisplatin, vincristine, methotrexate, bleomycin alternating with actinomycin D, cyclophosphamide and etoposide) failed to exhibit a significant correlation between cumulative cisplatin dose and chemotherapy-induced infertility [50]. Ejaculatory volume was decreased in all patients receiving a 5-day regimen of BEP for advanced stages of testis cancer [35]. Two to four cycles of BEP for 3 to 8 weeks rendered 53% and 44% of testicular cancer patients azoospermic and oligozoospermic respectively 6 months after stopping treatment. A significant decline in progressive sperm motility persisted up to 18 months among the same group. Moreover, the same study documented significant rises in FSH and LH concentrations after treatment [12].

#### Other Alkylating Agent Based

ChLVPP (chlorambucil, vinblastine, prednisolone, and procarbazine) chemotherapy caused extensive germinal epithelial damage and elevated FSH levels persisting up to 17 years after treatment [36]. Conditioning with BEAM (carmustine, etoposide, cytosine arabinoside and melphalan) combination allowed recovery of spermatogenesis in only 4 of 25 men with a mean follow up period of 40.2 months [37].

#### Non-Alkylating Agent Combinations

##### Doxorubicin Based

The first non-alkylating agent combination, ABVD (adriamycin, bleomycin, vinblastine and decarbazine), represented a breakthrough in anti-HD treatment regimens. 40% and 38% of HD patients became azoospermic and severely oligozoospermic respectively after 4 to 8 cycles of treatment with ABVD. Elevated FSH levels began returning to equal control levels after the 12th month [12] of follow up. However, the post-ABVD treatment sperm recovery rate of 90% within 1-5 years of treatment together with its high therapeutic efficacy, have made ABVD the standard HD treatment to date [28, 42].

##### Other Non-Alkylating Agent Based

NOVP (mitoxantrone, vincristine, vinblastine and prednisone) caused azoospermia in 38 % and severe oligospermia in 62 % after one month of treatment. Rapid recovery to normospermia occurred after 4.5 months of treatment completion in 63% suggesting only minimal germ stem cell toxicity [43].

#### **Anti-Cancer Agent Effects on Sperm Genome (Table 2)**

Mutations in mice germ cells after treatment with various agents such as chlorambucil, CY, etoposide, melphalan, mitomycin C, procarbazine and trophosphamide were found to take either of three forms; dominant lethal mutations that lead to embryonic death due to extensive chromosomal instability, heritable non-lethal translocations, and specific-locus mutations that allow development of the embryo but with the risk of carrying defective genomic material [11]. The risk of chemotherapeutic agents adversely affecting sperm chromatin material is highest during the early post-treatment period and declines after the first few weeks [11]. This represents the basis of current recommendation for a period of contraception or delay of sperm cryopreservation after therapy as a washout period before attempting conception [51]. Despite the abundance of animal studies that confirm these findings, recent studies on human cancer survivors showed inconsistent results with some authors observing significant increases in DNA damage in post treatment samples while

others did not [12, 18, 35, 52]. Controversial results underscore the necessity of further human research before the true adverse effect of anti-cancer agents on human germ cell genetic material is affirmed.

#### **Single Agent Effects**

##### Alkylating Agents

##### Mustards

Paternal CY administration was a cause of increased apoptosis in the embryo [53]. Lipid peroxidation, associated with a high frequency of single strand breaks in DNA, was almost doubled after CY treatment as detected by fluorimetric analysis of DNA unwinding (FADU) [54].

Dose-dependant chlormethine-induced dominant lethal and specific-locus mutations were noted in all spermatogenetic stages except stem cell spermatogonia [55].

Trophosphamide, which is structurally related to CY also induced chromosomal aberrations in 55% of the zygotes produced by trophosphamide-exposed mice spermatozoa and spermatids [56].

Similar to chlorambucil, melphalan-treated mice showed late stage spermatogenetic cell dominant lethal mutations and heritable translocations [58].

The significant levels of dominant lethal mutations induced by mustine were expressed as failure of development of embryonic trophoderm outgrowth and inner cell mass differentiation [59].

##### Organoplatinum Compounds

A dose-related adverse effect of cis-platinum on the chromosomal integrity of differentiating spermatogonia has been documented in male mice [24]. However, these aberrations expressed a decrease in transmission intensity as germ cells matured [60].

##### Chloroethyl Nitrosoureas

Carmustin (BCNU) induced a significant increase in spermatocyte chromosomal breakage [24]. Ehling et al documented a dose dependent relationship between BCNU and lomustine (CCNU) treatment, and dominant lethal and specific-locus mutation induction in post-spermatogonial germ cell stages [61].

##### Other Alkylating Agents

Low doses of busulfan induced similar specific-locus and dominant lethal mutation results in spermatozoa while higher doses affected spermatids [62]. Similar to mustine, significant levels of dominant lethal mutations were evident in procarbazine treated mice [59]. Among 14 agents, thio-TEPA was the only drug that induced significant chromosome translocations in spermatocytes [24]. Methyl methanesulfonate (MMS) treatment for 5 days resulted in a high frequency of germ cell apoptosis [25]. Mitomycin C increased frequencies of micronuclei in early phases of primary spermatocytic development [63]. Dacarbazine caused significant increases in heritable translocation rates reaching 2.13% compared to 0.05% in controls. This effect lasted for only 40 days after ending treatment suggesting that the affected spermatogenetic stages were the spermatids and early spermatozoa, rather than the germ stem cells [64].

##### Non-Alkylating Agents

##### Topoisomerase II Inhibitors

Etoposide produces peak mutagenicity in primary spermatocytic stages where recombinational events normally occur, leading to an increase in non-disjunction which poses a significant genetic risk in humans [67]. Merbarone together with etoposide induced significant increases in the frequencies of total hyperhaploid and diploid sperm in mice [68].

##### Radiomimetic Chemicals

In contrast to oocytes, male mice germ cells were insensitive to the mutagenicity of bleomycin, an interesting observation that was attributed to differences in the chromatin configuration between

**Table 2.** Anti-Cancer Agent Effects on Sperm Genome

Group Type	Subgroup	Anti-Cancer Agent	Effects on Sperm Genome	Ref.	
<i>Alkylating agents</i>	<u>Mustards</u>	Cyclophosphamide	<ul style="list-style-type: none"> <li>Apoptosis in the embryo</li> <li>High frequency of single strand breaks in DNA</li> </ul>	[53] [54]	
		Chlormethine	<ul style="list-style-type: none"> <li>Dominant lethal and specific-locus mutations in all spermatogenic stages except stem cell spermatogonia</li> </ul>	[55]	
		Trophosphamide	<ul style="list-style-type: none"> <li>Zygote chromosomal aberrations</li> </ul>	[56]	
		Chlorambucil	<ul style="list-style-type: none"> <li>Dominant lethal mutations and heritable translocations in later stages of spermatogenic cells</li> </ul>	[57]	
		Melphalan	<ul style="list-style-type: none"> <li>Dominant lethal mutations and heritable translocations in later stages of spermatogenic cells</li> </ul>	[58]	
		Mustine	<ul style="list-style-type: none"> <li>Dominant lethal mutations and failure of embryonic development</li> </ul>	[59]	
	<u>Organoplatinum compounds</u>	Platinol (cis-platinum)	<ul style="list-style-type: none"> <li>Chromosomal breakage and aberrations in differentiating spermatogonia</li> </ul>	[24, 60]	
	<u>Chloroethyl nitrosoureas</u>	Carmustine and Lomustine	<ul style="list-style-type: none"> <li>Significant increase in spermatocyte chromosomal breakage</li> <li>Dominant lethal mutations and specific-locus mutations in post-spermatogonial germ cell stages</li> </ul>	[24] [61]	
	<u>Other alkylating agents</u>	Busulfan	<ul style="list-style-type: none"> <li>Specific-locus and dominant lethal mutations in spermatozoa and spermatids with higher doses</li> </ul>	[62]	
		Procarbazine	<ul style="list-style-type: none"> <li>Dominant lethal mutations in offspring</li> </ul>	[59]	
		ThioTEPA	<ul style="list-style-type: none"> <li>Chromosome translocations in spermatocytes</li> </ul>	[24]	
		Methyl methanesulphonate (MMS)	<ul style="list-style-type: none"> <li>High levels of germ cell apoptosis</li> </ul>	[25]	
		Mitomycin C	<ul style="list-style-type: none"> <li>Increased frequencies of micronuclei in early phases of primary spermatocytic development</li> </ul>	[63]	
		Dacarbazine	<ul style="list-style-type: none"> <li>Increase in heritable translocation rate of spermatids and early spermatozoa</li> </ul>	[64]	
	<u>Agent combinations</u>	CHOP	<ul style="list-style-type: none"> <li>Increased apoptosis, increased abnormal DNA, and significant increase in pre and post-implantation fetal losses</li> </ul>	[29]	
		MOPP	<ul style="list-style-type: none"> <li>Increase in sperm aneuploidy</li> </ul>	[65]	
		BEP	<ul style="list-style-type: none"> <li>Increase in levels of DNA fragmentation</li> <li>Increase in the frequency of disomy sperm for chromosomes 12, X and Y</li> </ul>	[35] [66]	
			<ul style="list-style-type: none"> <li>Increase in diploidy and disomy for chromosomes 16, 18, and XY</li> <li>Decrease in DNA fragmentation levels</li> </ul>	[51] [52]	
	<i>Non-Alkylating agents</i>	<u>Topoisomerase II inhibitors</u>	Etoposide (VP-16)	<ul style="list-style-type: none"> <li>Increase in nondisjunction in primary spermatocytic stage</li> <li>Increase in the frequencies of total hyperhaploid and diploid sperm</li> </ul>	[67] [68]
			Merbarone (MER)	<ul style="list-style-type: none"> <li>Increase in the frequencies of total hyperhaploid and diploid sperm</li> </ul>	[68]
<u>Radiomimetic chemicals</u>		Bleomycin	<ul style="list-style-type: none"> <li>Induction of multi-locus deletions in stem cells and differentiating spermatogonia</li> </ul>	[69]	
<u>Aneuploidy inducers</u>		Vinblastine	<ul style="list-style-type: none"> <li>Increased frequencies of hyperploidy in secondary spermatocytes</li> </ul>	[70]	
<u>Antimetabolites</u>		6-Mercaptopurine	<ul style="list-style-type: none"> <li>Dominant lethal mutations in late spermatogonial and early spermatocytic stages</li> </ul>	[71]	
		Fludarabine	<ul style="list-style-type: none"> <li>Extensive DNA damage</li> </ul>	[41]	
		5-flourouracil	<ul style="list-style-type: none"> <li>Increase in chromosomal aberrations</li> </ul>	[72]	
<u>Agent combinations</u>		NOVP	<ul style="list-style-type: none"> <li>Increase in sperm sex chromosome aneuploidies</li> </ul>	[73]	
		MACOP-B	<ul style="list-style-type: none"> <li>No significant increase in structural chromosomal abnormalities</li> </ul>	[74]	

sexes implying gender-specific safety to certain agents [75]. Others challenged this, noting that bleomycin had a mutagenic effect on male germ cells by inducing multi-locus deletions and affecting both germ stem cells as well as differentiating spermatogonia [69].

#### Aneuploidy Inducers

Vinblastine increased frequencies of hyperploidy in secondary spermatocytes [70] although others have suggested variations were

not significant [76]. While vincristine sulfate reduces fertilization ability, the subject of genetic based germ cell damage remains controversial as some have reported damage [40]. while others have not observed such damage [77].

#### Antimetabolites

Information on antimetabolite-induced sperm DNA damage remains sparse. 6-mercaptopurine was unique for inducing dominant lethal mutations in late spermatogonial and early spermatocytic stages [71]. Extensive sperm DNA damage was detected after the 7th month of fludarabine treatment [41]. 5-flourouracil also caused chromosomal aberrations in mammalian germ cells [72].

#### **Combination Treatment Effects**

##### **Alkylating Agent Combinations**

##### Cyclophosphamide Based

CHOP increased germ cell apoptosis and DNA abnormalities with a significant increase in pre and post-implantation fetal losses [48].

##### Procarbazine Based

MOPP-associated aneuploidy in HD patient samples was double that detected in donor samples [65].

##### Platinum Based

The baseline frequency of sperm disomy increased by over 100% for chromosomes 12, X and Y during and immediately post-BEP therapy, [66] which contradicted former studies by the same group that failed to detect such a significant correlation [78]. Similar results were reported for chromosomes 16, 18, and XY [51]. All patients who had received BEP on a 5-day regimen for advanced stages of testis cancer showed significant increases in levels of DNA fragmentation comparing their pre and post-treatment semen samples [35]. Interestingly, Stahl and his group documented a decrease in DNA fragmentation levels after treatment with 3 or more cisplatin-based cycles [52].

##### **Non-Alkylating Agent Combinations**

Multi-color fluorescence in situ hybridization (FISH) evaluation of HD patients' sperm exposed to NOV (Novanthrone, Oncovin, Vinblastine, Prednisone) demonstrated up to a 14 fold increase in a variety of genomic numerical abnormalities sperm that resolved 1-2 years after treatment [73]. No significant difference in structural chromosomal abnormalities was detected in MACOP-B treated patients compared to control donors 3 years post-treatment [74].

### **STRATEGIES TO MAXIMIZE FERTILITY POTENTIAL**

Reviewing the harmful side effects of the different anti-cancer agents emphasizes the undesirable byproducts of these therapies and highlights the need for new efforts to minimize gonadotoxicity. Evolving strategies seek to minimize the adverse fertility impact of these agents while maintaining their clinical efficacy.

#### **Regimen Modification**

Contemporary clinical trials have focused on dose reduction, alternative regimens, and surveillance protocols to reduce the drug-related toxicity without compromising cure rates [79]. Treatment choices are tailored according to pretreatment clinical criteria such as tumor pathology, primary tumor extension, metastatic presence and serum tumor marker levels [80]. Chemotherapeutic regimens may be modified by either reducing agent dosage or frequency of administration, or by substituting agents with less gonadotoxic alternatives. Administration of a chemotherapeutic drug in weekly fractionated doses rather than a single dose allowed genetic repair resulting in a reduction of its mutagenicity [14]. One of the first examples for agent substitution was the breakthrough implication of the first non-alkylating agent-containing combination ABVD for the treatment of HD. Comparing alkylating agents to nonalkylating

agents, ABVD not only allowed a sperm recovery rate of 90% post-treatment but improved the 5 year recurrence-free survival by 10% over standard MOPP regimens [42, 81]. FSH rose in 60% of those receiving alkylating agent chemotherapy such as MOPP, MOPP/doxorubicin, bleomycin, vinblastin (ABV) and BEACOPP in contrast to only 8% of patients receiving nonalkylating agents such as ABVD and EBVP (epirubicin, bleomycin, vinblastin and prednisone). Spermatogenesis recovered in 30% versus 82% in both groups respectively [82]. Newer lines of therapy such as the inhibitors of the epidermal growth factor receptor or the vascular endothelial growth factor hold promise to avoid the adverse affects of current conventional cytotoxic therapy, although the impact on spermatogenesis remains to be established.

#### **Sperm Cryopreservation**

Sperm cryopreservation has been established as the safest and most effective method for preserving fertility in young cancer patients in advance of gonadotoxic therapy. Assisted reproductive techniques (ARTs) particularly intracytoplasmic sperm injection (ICSI) enabled conception with the least requirement of sperm quantity and quality. The use of cryopreserved sperm in IVF (in vitro fertilization)/ICSI produces comparable, if not superior results to fresh sperm in terms of both fertilization and pregnancy rates [83]. Applying these technologies to cancer survivors resulted in fertilization rates of over 72% and delivery rates approaching 50% [84, 85]. Recent data on the sperm DNA integrity have been promising providing additional reassurance for the use of these techniques in cancer patients [18, 52]. In cases of pretreatment azoospermia, sperm may be collected either from the ejaculate, the epididymis by aspiration or the testis by testicular extraction (Onco-TESE). With Onco-TESE, which is pretreatment testicular extraction of sperm, sperm retrieval was possible in nearly half the cases of malignant lymphoma and testicular cancer that suffered azoospermia without delaying post-operative adjuvant therapy [86]. This technique was believed to be superior to conventional post-treatment TESE by avoiding the extraction of genetically damaged sperm after chemotherapy [86].

Despite the effectiveness of sperm cryopreservation in preserving fertility, less than 18 % of newly diagnosed male cancer patients used this technology [85]. In a recent study of 522 patients with non-germ cell urological cancer, only 32.1% desired pretreatment sperm banking. On multivariate analysis, aging and previous fatherhood maintained inverse associations with the wish to bank sperm prior to treatment [87]. This option remains underutilized due to a variety of factors including failure of physicians to offer semen banking while counseling their patients in advance to gonadotoxic therapy [4], suggesting that many physicians may be unfamiliar with current preservation options [88]. In addition to the costs of sperm banking, finding proper facilities and specialists for fertility preservation remains problematic [88-91]. Only 60% of young cancer survivors recalled being counseled about their fertility before therapy while only 51% were offered sperm banking [5]. Poor health status and disease prognosis correlated inversely with occurrence of proper fertility counseling [88]. Lack of proper guidelines due to unresolved medical, legal and ethical issues were other proposed reasons for this deficiency in proper counseling [92]. The British Fertility Society recommendations stated that sperm banking was to be an option for every pubertal male cancer patient who carried a medium to high risk of fertility impairment [93]. Several years later, the American Society of Clinical Oncology (ASCO) emphasized the importance of providing a thorough disclosure of the risks of future infertility in addition to a comprehensive discussion of all available options for fertility preservation to all cancer patients antecedent to commencing their treatment [81]. Oncologists were found to be the major motivators for these patients to bank their sperm [94].

### Potential Therapeutic Options

The past decades have witnessed intense research effort focusing on cytoprotective strategies to alleviate the harmful effects of cancer treatments on fertility. Strategies to optimize fertility can be employed before treatment begins, during therapy, or after treatment has been received.

#### *Hormonal Manipulation*

Early studies demonstrated that chemotherapeutic agents are more likely to cause gonadal damage when administered in the post-pubertal age as opposed to the pre-pubertal age which may be explained by the contrast in degree of gonadal activity between both periods [44]. Based on this principle, early animal studies focused on mimicking the state of insensitivity of the pre-pubescent testis to gonadotoxicity by creating an artificial state of spermatogenic quiescence within the testes. Prophylactic down-regulation of the pituitary gland by the negative feed-back effect of either gonadotropin releasing hormone (GnRH) agonists or testosterone administration before cytotoxic therapy would suppress active spermatogenesis theoretically rendering the testes less susceptible to cytotoxic therapy [95]. Germ cell repopulation indices were significantly higher after using a combination of a GnRH antagonist (Nal-Glu) and an antiandrogen (flutamide) for periods as short as 2 weeks prior to procarbazine therapy. Longer periods have been recorded in order to achieve germ cell protection with testosterone and estradiol treatment [96]. Nevertheless, these techniques were proven of value in animals only and have not been successful in humans. In addition to the prophylactic role of GnRH agonists in the preservation of spermatogenesis, further studies on animals have shown a therapeutic role for GnRH analogues in the rapid recovery of spermatogenesis if given during or shortly after cytotoxic treatment. When GnRH treatment was given for the first 10 weeks immediately after procarbazine injection in the rat model, germ cell population remained intact in 98% and almost 90% after 10 and 20 weeks respectively following treatment despite the cessation of GnRH agonist therapy [95]. Shetty and Meistrich hypothesized that the gonadotoxicity of cancer treatment led to significant increases in testosterone and FSH levels resulting in inhibition of spermatogonial differentiation. Therefore suppressing these levels using GnRH agonists or antagonists would potentially induce early recovery of spermatogonial differentiation [6]. Others succeeded in achieving similar results using three doses of the GnRH agonist, leuporelin after busulfan treatment [97]. Studies on germ cell transplantation by Ogawa and his group reported a positive impact of applying the same technique to busulfan treated recipient testes before transplanting germ stem cells, suggesting hormonal suppression to be a potential adjunct to this novel technology [98]. The discovery of this post cytotoxic treatment restorative role raised a debate on the actual mechanism by which GnRH agonist therapy protected germ cells. While some have suggested that the spermatogonial differentiation block caused by cytotoxic therapy was due to damage on the somatic cell level rather than the germ cell [99], others suggested both, germ cell and somatic cell damage as causative [6]. Despite the success in animal models, these cytoprotective strategies have not been proven effective in humans what may be explained by species specific differences in the gonadal response [18]. At the present time, cytoprotective techniques are not considered as effective options for fertility preservation.

#### *Prepubertal Germ Cell and Gonadal Tissue Cryopreservation, Transplantation and In Vitro Maturation*

Another technique, that holds promise for the preservation of future fertility of prepubertal patients receiving chemotherapy, is the removal and cryopreservation of their gonadal tissue that normally contains diploid germ stem cells prior to therapy. Using dimethyl sulphoxide (DMSO) as a cryoprotectant, a recent study was able to achieve excellent results in terms of preserving the architecture, viability and function of the major testicular components after

cryopreserving immature testicular tissue from prepubertal boys on the verge of receiving cytotoxic therapy [100]. Subsequently after completion of anti-cancer therapy, mature haploid spermatozoa may be potentially attained by either autotransplantation of this material into the patient's testis or xenotransplantation in cases of bilateral testicular compromise using different species as a reservoir site where complete spermatogenesis could take place. Numerous studies on different species have proved the success of these approaches in achieving mature spermatozoa, some of which resulted in full term pregnancies [98, 101]. In contrast, similar studies on human germ cell xenografting failed to progress beyond the stage of germ cell proliferation [102-104] which may be attributed to the species specificity of the germ cell supporting system, namely the Sertoli cells [13]. To overcome this obstacle, a second option is to transplant whole testicular tissue containing germ cells with their supporting niches into ectopic sites. Despite proof of concept in mice [105], successful human application has remained challenging [105, 106]. While research strives to prove the success of human stem cell transplantation, the key challenges continue to be the optimal retrieval and transfer technique to allow high sperm recovery while minimizing the risk of transplanting a tumor-cell contaminated sample or transmitting zoonotic diseases in cases of xenotransplantation [9]. Researchers have tried to achieve human germ cell maturation by in-vitro germ cell differentiation using special cultures, an alternative approach that is still in its trial phase [107]. The first in-vitro derived human sperm was reported recently by Nayernia and his team [108]. By using special cultures, they were able to culture stem cells, derived from a male embryo, into mature haploid sperm. While this novel technique holds promise, the full potential of the human embryonic stem cell derived germ cells and sperm remains to be tested.

With the aid of assisted reproductive technology, minimal amount of mature sperm produced by these techniques will allow cancer survivors to father children. Until these techniques become applicable on humans, cryopreservation of prepubertal testicular tissue may be the only bridge to these forthcoming technologies and therefore should be offered to all young cancer patients about to receive anti-cancer treatment.

#### *Cytoprotective Adjuvants*

Pre-treating subjects with special cytoprotective substances prior to receiving chemotherapy may offer the potential to reduce the adverse gonadotoxic effect of several anti-cancer agents via various mechanisms. Albino rats treated with lipoic acid 1 day prior to ADR administration reversed the negative biochemical and morphological changes induced by ADR maintaining enzymatic homeostasis within the testis and causing a 66% increase in sperm count and 56% increase in sperm motility [38]. Similarly, treatment with lycopene prior to ADR treatment preserved sperm motility and morphology in addition to reversing adverse testicular and epididymal histopathological changes [39]. Another group, using the same method prior to CY treatment, confirmed the protective efficacy of lipoic acid on semen quality and testicular histology integrity. Moreover, the antioxidant effect of lipoic acid reduced semen ROS levels and sperm DNA damage over levels observed in the CY group [4, 109]. Incorporating CY with Yukmijihwang-tang (YMJ), a historical herbal anti-aging medicine used for centuries in Asian countries, was proven to inhibit lipid peroxidation, restore a crucial transcription factor in germ cell differentiation and improve sperm counts and motility [17]. Co-administration of another established herbal antioxidant, Satureja khuzestanica essential oil (SKEO), significantly improved CY-impaired plasma testosterone levels and sperm quality, doubling the pregnancy rates seen when CY was used alone [110]. A significant improvement in average testicular weights, sperm count and morphology and sperm DNA integrity documented by TUNEL and comet assays (22.88 % of DNA in the tail vs. 29.52 % without cytoprotection) proved Astaxanthin, a carotenoid pigment, to be an effective chemoprotective adjuvant to CY

[111]. Pretreating male mice with folic acid for 4 days prior to methotrexate administration followed by a single dose of folic acid significantly enhanced sperm counts and morphology and reduced sperm DNA damage [112]. Although these studies have succeeded to report the protective role of cytoprotective agents against the gonadotoxicity of chemotherapy, yet the influence of these agents on the efficacy of chemotherapy is a question that awaits further studies to be answered.

### OFFSPRING HEALTH WELFARE

With the potential for anti-cancer agents to induce extensive damage to sperm genetic material which does not obviate the fertilizing ability of sperm through assisted reproduction, there exists a theoretical risk of inducing pregnancies with genetically impaired sperm. As the degree of sperm DNA damage in both animals and men was directly correlated to the failure of embryo development [113], an increase in spontaneous abortion rate after chemotherapy would be inferred. However, this correlation was refuted in another study [114]. In contrast to animal studies during the past 2 decades that documented transgenerational effects of cytotoxic therapy particularly with CY and ethylnitrosourea [115], epidemiological studies have failed to detect clinical evidence for heritable damage in the form of congenital malformations or childhood cancer [116, 117]. Offspring of cancer patients have birth outcomes resembling the general population with regards to birth weight, sex ratios and major birth defects [118]. A large retrospective study of 2,323 pregnancies did not identify adverse pregnancy outcomes after treatment with most anti-cancer agents [119]. After excluding hereditary cases of chromosomal abnormalities in the offspring of a Danish childhood cancer survivor population, abnormal karyotypes were detected in only 0.21% of their progeny including aborted cases resembling the incidence among children of their cancer-free siblings. The incidence of Trisomy 21 and Turner syndrome were similar in both groups [120].

Although the bulk of the studies concerned with this issue seem to be reassuring, a meticulous analysis of the data regarding the welfare of cancer survivor progeny may reveal some methodological flaws. For example, most studies lack adequate population numbers, include a selective choice of drugs and do not report assisted reproduction outcomes where natural fertilization is bypassed. Therefore, it is crucial to counsel cancer survivors approaching assisted reproduction on the nascence of these issues as we await larger longitudinal studies of modern chemotherapeutic agents to establish optimal knowledge of the hazards of these agents on offspring welfare. To minimize the reproductive hazard, a washout period of several spermatogenic cycles has been advocated before attempting conception based on the observation that aneuploidy may persist beyond 18 months post-chemotherapy [51].

### CONCLUSION

The advent of anti-cancer agents has led to substantial improvements in survival rates with many cancer patients approaching that of the normal population. This improvement has been shadowed by equivalent concerns about future fatherhood potential of cancer survivors and the welfare of their progeny.

Studies have shown that almost all anticancer agents exhibit a gonadotoxic effect that varies according to the spermatogenic stage targeted by the agent, the type of agent or agent combination, and both the dosage and frequency of agent administration. Research has documented this adverse effect to extend beyond bulk semen parameters breaching sperm genomics which raised a new concern about transferring damaged genetic material to offspring. Although studies have failed to show an increase in the incidence of congenital abnormalities in children born to cancer-survivors, low population numbers and methodological limitations render the long term health implications of chemotherapy on offspring unclear.

It is incumbent on the multidisciplinary team of oncologists and infertility specialists to properly counsel young cancer patients with the current data regarding their disease, their potential fertility before and after cancer treatment, and the established strategies for fertility preservation with emphasis on sperm cryopreservation, in addition to the contemporary data on the long term outcomes of their conception.

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