Chemotherapy and Male Reproduction

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Abstract

Previous studies have demonstrated adolescent and adult chemotherapy can impact later life human male and female fertility, and promote epigenetic alterations in the sperm. Animal rat studies have shown pubertal male chemotherapy exposure can promote DNA methylation alterations in adult sperm and promote epigenetic transgenerational inheritance of sperm epigenetic alterations and adult onset disease for future generations. Studies in humans also demonstrate epigenetic impact on fertility and sperm. Sperm differential DNA methylation regions (DMRs) were identified in control and chemotherapy adolescent exposure population comparisons. These chemotherapy-induced DMR alterations have the capacity to impact the individual exposed and future offspring and subsequent generations' health, suggesting cryopreservation of sperm and eggs prior to chemotherapy should be considered when possible.

Key Points

- Chemotherapy induced sperm DNA methylation facilitates epigenetic inheritance.
- Chemotherapy promotes epigenetic transgenerational inheritance of pathology.
- Pubertal exposure to chemotherapy impacts later life sperm epigenetics and genetics.
- Cancer chemotherapy treatment needs to consider later life and generational impacts.

Introduction

Anticancer chemotherapies are often vital to the survival of people with cancers. However, these same chemotherapeutic drugs carry a risk of inducing infertility in treated patients. In human males, chemotherapy can affect the somatic cells of the testis, such as Sertoli cells or Leydig cells, leading to impairment of testosterone production or of inhibin – luteinizing hormone (LH) signaling, which in turn can interfere with normal sperm production (Delessard *et al.*, 2020; van Santen *et al.*, 2020). Chemotherapeutic drugs can also affect developing germ cells such as spermatogonia, spermatocytes and spermatids, resulting in impaired sperm production and either low or zero sperm counts (oligospermia or azoospermia). If some or all of the spermatogonial stem cells are destroyed, then future fertility may be permanently decreased or abolished (Delessard *et al.*, 2020; Pasten Gonzalez *et al.*, 2024; van Santen *et al.*, 2020). Adult males would show the gonadotoxic effects of chemotherapy relatively quickly, but pubertal or pre-pubertal boys treated for cancers might show sub-fertility or infertility only years later, after the normal time of puberty, if the stem cell populations for germ cells or testicular somatic cells were compromised.

In females, as opposed to males, new germ cells are not made during adulthood. Rather, there is a pool of oocytes, stored in primordial follicles, that are arrested in the early stages of meiosis. A few follicles at a time leave the arrested pool, providing oocytes for ovulation during adulthood (Kezele *et al.*, 2002). If the pool of primordial follicles is damaged or depleted early, then infertility occurs, often associated with a premature menopause-like condition called premature ovarian insufficiency (McGlacken-Byrne and Conway, 2022). Anticancer chemotherapies can damage the oocyte pool in several ways (Mauri *et al.*, 2020). They can cause accelerated maturation of ovarian follicles by inducing apoptosis and atresia of the fast-growing antral follicles, which in turn stimulates faster recruitment from the arrested primordial follicle pool. They can directly stimulate the PI3K/PTEN/Akt signaling pathway in follicles, which will promote activation and development of primordial follicles. Chemotherapeutics can also directly damage the DNA in the oocytes of arrested primordial follicles, leading to oocyte apoptosis. And they can disrupt ovarian vascularization, leading to reduced blood flow and cellular damage related to ischemia (Mauri *et al.*, 2020). Adult females receiving chemotherapy may show an immediate post-treatment amenorrhea, with some women later recovering ovarian cyclicity, although often for a shorter duration of their lives before experiencing premature ovarian insufficiency (van Santen *et al.*, 2020). Pre-pubertal girls receiving chemotherapy can be at risk of depleting their primordial follicle pool, and so later would have no growing follicles to produce estrogen, therefore needing hormone replacement therapy at the time of puberty (van Santen *et al.*, 2020).

Chemotherapeutic drugs differ in how toxic they are to gonads and germ cells, with alkylating agents (for example cyclophosphamide, cisplatin, carboplatin, chlorambucil, and melphalan) being most often cited as having the greatest gonadotoxicity (Bhardwaj *et al.*, 2023; Delessard *et al.*, 2020; Mauri *et al.*, 2020; Pasten Gonzalez *et al.*, 2024; van Santen *et al.*, 2020). However, anticancer chemotherapy regimens seldom use only one drug, and overall gonadotoxicity and future fertility depend more upon combinations of therapy factors, such as the number of cycles of treatment, the co-occurrence of radiation therapy, the specific drug combinations used, and the ages of the patients treated (Bhardwaj *et al.*, 2023; Delessard *et al.*, 2020; Mauri *et al.*, 2020; van Santen *et al.*, 2020). For example, women 40 years old or older receiving a breast cancer chemotherapy regimen of six cycles of CMF, (cyclophosphamide, methotrexate, fluorouracil) have a high risk (> 80%) of developing infertility, while women 30 years old or younger who receive the same CMF treatment have a low (< 20%) risk of infertility (Lee *et al.*, 2006).

People at risk of developing infertility from anticancer therapies are encouraged to have sperm or eggs collected and stored (Pasten Gonzalez *et al.*, 2024; van Santen *et al.*, 2020). For pre-pubertal girls, it is possible to cryopreserve a section of ovarian cortex for future collection of oocytes (Bhardwaj *et al.*, 2023). Similar strategies are not yet clinically available for pre-pubertal boys (Bhardwaj *et al.*, 2023; van Santen *et al.*, 2020).

Chemotherapy

Our previous studies demonstrated adolescence chemotherapy exposure promoted, later in life, an epigenetic DNA methylation alteration in human and animal model sperm (Shnorhavorian *et al.*, 2017). Recently, we found in a rat model that pubertal chemotherapy exposure promoted epigenetic modification in sperm for the male exposed later in life, but also in the male's offspring transgenerationally (F3 generation) later (Thompson *et al.*, 2022). In addition to the sperm epigenetic alterations, a variety of pathologies (e.g., obesity, kidney, and prostate) increased in offspring and grand-offspring adult male rats (Thompson *et al.*, 2022). Therefore, adolescent chemotherapy exposures not only impact adult sperm epigenetic alterations, but promote the epigenetic transgenerational inheritance of disease in subsequent generations (Shnorhavorian *et al.*, 2017; Thompson *et al.*, 2022). Similar observations of chemotherapy on female reproduction have been observed. This epigenetic inheritance is dependent on the transmission of altered epigenetics through the germline (i.e., sperm) to subsequent generations.

Although the efficacy of the various chemotherapies for osteosarcoma survivors has been reported, the impacts on sperm epigenetics has not been previously investigated. Studies are needed to expand our previous study of the impacts of adolescent chemotherapy on adult sperm epigenetics (Shnorhavorian *et al.*, 2017). Variables such as chemotherapy regimens, absence or presence of ifosfamide, or various demographics or age need to be assessed.

Previous alterations in differential DNA methylation regions (DMRs) were determined with methylated DNA immunoprecipitation (MeDIP) followed by next generation sequencing analysis in response to chemotherapy. The impact of the sperm epigenetic alterations observed on the individuals and their subsequent generations is discussed.

Discussion

An initial study of chemotherapy effects on epigenetics that involved nine control patients versus nine survivors of adolescent chemotherapy identified a later life impact on sperm epigenetics (Shnorhavorian *et al.*, 2017). A significant impact on differential DNA methylation regions (DMRs) in the sperm epigenetics was identified in the chemotherapy patients compared to the control population male sperm. A more recent study using an animal (i.e., rat) model used adolescent exposure to the chemotherapy ifosfamide followed by breeding adults for three generations to obtain the F1, F3, and F3 male rats for analysis of epigenetic impacts on the sperm and subsequent transgenerational disease (Thompson *et al.*, 2022). Observations demonstrate adolescent chemotherapy can promote permanent alterations in sperm DNA methylation for the individual exposed and all subsequent generations through epigenetic transgenerational inheritance (Thompson *et al.*, 2022). These observations suggest chemotherapy exposure of males can modify the epigenetics in the spermatogonia stem cell in the testis to lead to sperm with epigenetic modifications that can be passed to subsequent generations through epigenetic transgenerational inheritance (Nilsson *et al.*, 2022). Similar observations on female reproduction following chemotherapy have been observed (Kezele *et al.*, 2002; Mauri *et al.*, 2020; McGlacken-Byrne and Conway, 2022).

All the chemotherapies and various regimens during adolescence have the capacity to promote an epigenetic alteration that, later in life, is passed through chemotherapy induced epigenetic transgenerational inheritance of germline effects and disease susceptibility to subsequent generations (Nilsson *et al.*, 2022). Similar to animal studies, chemotherapies such as ifosfamide can promote epigenetic transgenerational inheritance in the offspring, grand-offspring, and great-grand offspring and beyond (Thompson *et al.*, 2022).

Adolescent or pubertal exposure to environmental toxicants has an increased capacity to impact the male germline due to the initiation of spermatogonia germ cell development at the onset of puberty. The spermatogonial germ cell population development initiates the spermatogenic process following endocrine induction during puberty (Law and Oatley, 2020). Therefore, the germ cells are more sensitive to environmental exposures to toxicants or therapeutics like chemotherapy (Ben Maamar *et al.*, 2021; Guerrero-Bosagna and Skinner, 2014). Although these exposures can also cause systemic impacts on all somatic cells and tissues to promote pathology in the individual exposed, it is the germline that is the critical cell to transmit

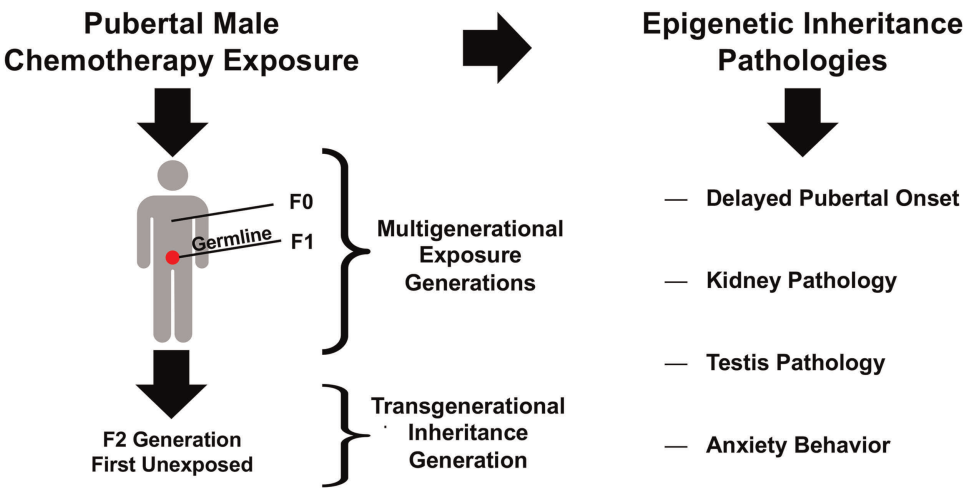

Fig. 1 Contrasting multigenerational and transgenerational exposures. Modified from Nilsson *et al.* (2018).

these effects through sperm to the subsequent generations (**Fig. 1**) (Nilsson *et al.*, 2022). This environmentally induced epigenetic transgenerational inheritance is germline mediated through epigenetic changes. Therefore, the pubertal chemotherapy exposure permanently alters the germline stem cell epigenetics to then, upon reproduction, be passed to the offspring and subsequent generations in a similar manner (Thompson *et al.*, 2022). These germline mediated epigenetic transgenerational inheritance processes have been shown to impact most biological systems and processes (King and Skinner, 2020; Nilsson *et al.*, 2018). Therefore, the chemotherapy may not have significant impacts on the individual exposed, but be passed on to subsequent generations through the germline to have significant impacts on health (**Fig. 1**) (Nilsson *et al.*, 2022). The rodent animal model (rats) used ifosfamide exposure during puberty to induce epigenetic transgenerational inheritance of health impacts of three subsequent generations following the F0 generation adolescent male chemotherapy (ifosfamide) exposure. Observations suggest adolescent chemotherapy exposure could have major health impacts on subsequent generations.

Conclusion

The current literature does not suggest chemotherapy should not be used, but it has been demonstrated that potentially more impactful consequences of the use of chemotherapy exist. Clearly the cancer treatment is needed and allows the individual treated to survive. However, the knowledge that chemotherapy exposure impacts the future generations' health does have ethical considerations and needs to be considered. The suggestion is that cryopreservation of sperm be considered so when an individual considers reproduction, the cryopreserved sperm, not having the epigenetic consequences of chemotherapy exposure, can be used. This will reduce the impacts on future generations' health. Cryopreservation of both sperm and eggs is feasible and been shown as an alternative when environmental exposures like chemotherapy are used or occur. Research supports that adolescent chemotherapy exposure can impact the germline epigenetics permanently to be passed upon reproduction to subsequent generations and impact future generations' health. Therefore, the concerns of environmentally induced epigenetic transgenerational inheritance on subsequent generations needs to be considered for the well-being and health of our future generations (Kabasenche and Skinner, 2014; Korolenko *et al.*, 2023).

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