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Effects of chemotherapeutic treatment on female reproductive function

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Summary

As a consequence of the improvement in diagnosis and therapy, many more young women will survive their cancer. For some of them, due to toxic treatments, there is the risk of compromising their reproductive function. There is a growing body of evidence about the specific effects of different chemotherapy drugs on the ovary, based on *in vitro* methods, complementing clinical information regarding ovarian function. This information, highlighting the varying effects of these drugs on the different cells and tissues of the ovary, is reviewed here.

KEY WORDS: chemotherapy, ovary, fertility, preservation, follicle.

Introduction

Fertility lifespan is directly correlated with the number of quiescent primordial follicles present in the ovary. Around the time of birth, this ovarian reserve contains around a million such follicles. The number then steadily declines until on-

ly around a thousand remain, at which time the menopause occurs, at the average age of 50 years, with fertility rates declining over the previous two decades (1, 2). In developed countries, due to both economic and social factors, motherhood is becoming progressively postponed to an age at which fertility is naturally declining. This average older age for childbearing is matched with a significant increase in the incidence of cancer (3). This means that every year many more women will be diagnosed with a new cancer during a fertile age, when they may have not yet started or at least completed their families. The effectiveness of cancer treatment has however greatly improved, especially for childhood cancers. With longer term survival rates so much better, increasing importance is now placed on attaining a good quality of life after treatment. Effects on fertility are key amongst factors affecting this, and have been acknowledged as a major stressor for cancer survivors (4, 5). Exposure to highly cytotoxic treatments during chemotherapy could negatively affect reproductive function via several possible pathways, primarily the ovary, but also potentially the hypothalamic-pituitary system or the uterus (Figure 1). It is clear that the ovary is the prime site of adverse effects of chemotherapy on female reproductive function, so this review will focus primarily on this aspect.

Chemotherapy-induced premature ovarian insufficiency

The principal and most vulnerable targets of chemotherapy within the female reproductive system are the ovaries, the functional units of which are the ovarian follicles. Each ovarian follicle is composed of a single female germ cell, oocyte, arrested in prophase I of the first meiotic division and surrounded by supportive somatic cells, the granulosa cells (GCs). Oocytes surrounded by a single layer of flattened GCs form the quiescent primordial follicle

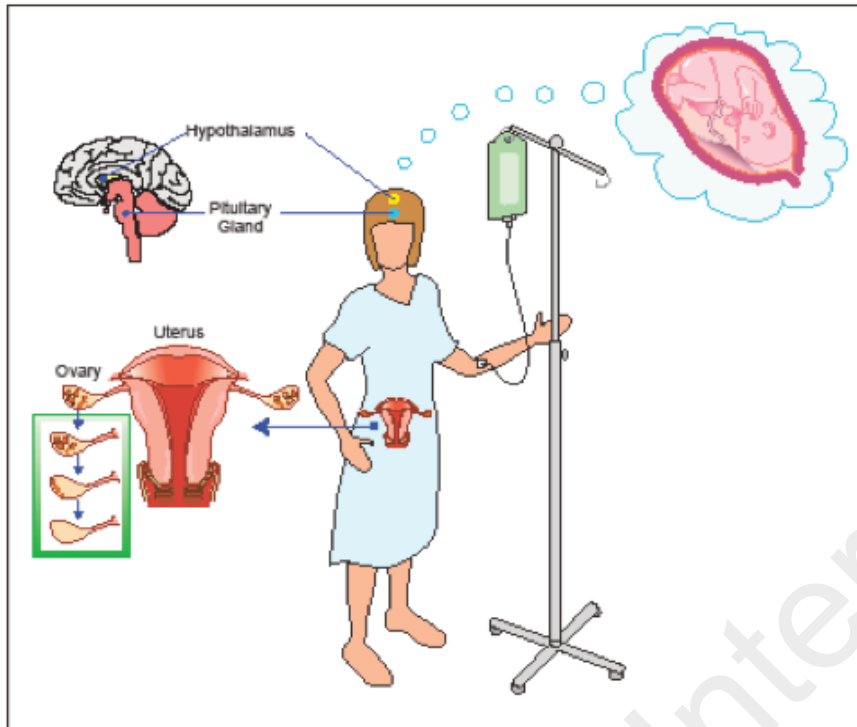


Figure 1 - Chemotherapeutic drugs are effective against malignant cells, but can also have an adverse impact on healthy tissues. When this damage occurs to the reproductive system, it is often evident in the ovaries, where the follicle reserve may be destroyed. In addition, drug toxicity could induce dysfunction in other organs, such as the uterus or the hypothalamus and pituitary gland. The possible decrease of reproductive potential generates great concerns about motherhood among cancer survivors.

(PF) pool. This store of resting follicles is formed before birth, leading to a limited and irreplaceable supply of female germ cells by birth. Every day, a tiny fraction of the remaining PFs are activated to leave the resting state and undergo growth initiation. The subsequent process of growth and maturation involves nuclear and cytoplasmic oocyte maturation as well as modifications in GCs, which become actively replicating cuboidal cells, stratifying into multiple layers that interact with the oocyte through paracrine signalling. Very few activated follicles will fully mature to ovulate, with the majority instead undergoing atresia, thus there are progressively fewer follicles at each stage of growth.

The cytotoxic effect of chemotherapy could damage a variety of cell types within the ovary, acting directly on follicles and/or on the surrounding stromal or vascular tissue. Similarly, within follicles, drugs could act primarily on the oocyte and/or on somatic granulosa or possibly theca cells. Any of these could lead to a reduction of gonad functionality through a depletion of the follicle number or interference with nor-

mal growth. Currently, one important hypothesis as to how loss of developing follicles results in depletion of the ovarian reserve is that damage to developing follicles results in increased activation of the resting PFs, such that they, in turn, will be in the growing stage of follicle development during subsequent chemotherapy doses, leading to a resulting dramatic decrease in PF numbers after several cycles of treatment: this is sometimes termed the 'burnout' theory (6). The degree of ovotoxicity varies with the therapeutic regime, but will also produce different age-dependent outcomes. In women near to menopause, iatrogenic reduction of the ovarian reserve will be more immediately evident because of the already naturally depleted population of follicles; in such women, menopause is increasingly likely immediately or shortly after chemotherapy. In younger women, after a certain period of amenorrhea, fertility could well be restored for several years, giving a reproductive window to cancer survivors. Nonetheless, menopause could then occur precociously, potentially not leaving enough time for family planning and also affecting the woman's long-

term health (7). Menopause usually occurs around the age of 50: when it occurs before the age of 40, it is termed premature ovarian insufficiency (POI) (8, 9). POI is related to a higher incidence of osteoporosis as well as to cardiovascular disorders, which could in turn increase mortality rates (10).

When a cancer therapy is administered to a child or prepubertal girl, there is potentially a very wide time interval before the appearance of symptoms of its deleterious action, from immediately to perhaps 2 or 3 decades later. One major issue in the assessment of chemotherapeutic damage on fertility results from this, namely the time required for thorough follow-up. A follow-up study should ideally examine aspects of fertility such as pregnancy, childbirth and timing of menopause. However, obtaining data on such end points would require following patients for decades. When viewed in this way, at this point very few studies carried out can truly be considered long term although the US base Childhood Cancer Survivor Study (CCSS) is a major example and has resulted in a wealth of data [for example (11-13)]. Others have also contributed to this (14) but most studies use more short-term, indirect markers of fertility (reviewed in 9). To further complicate the situation, chemotherapy-induced amenorrhea (CIA) is the most widely used marker of POI, but its definition varies between studies in terms of period of onset and duration. More importantly, though, neither its occurrence, nor its subsequent reversion, is equivalent to infertility/fertility respectively.

Anti-Müllerian hormone (AMH) level has proved the most reliable, albeit indirect, marker of the ovarian reserve (15). AMH blood levels predominantly derive from small antral follicles, mainly 5-8mm in diameter (16). In contrast with other hormonal markers (e.g. FSH and estradiol), AMH levels do not vary significantly throughout the menstrual cycle. Furthermore, AMH concentration appears to be a good indirect marker of the ovarian reserve, with levels falling as women approach menopause or when ovotoxic treatment is applied (17). The peak in serum AMH is at age 24, with a progressive rise through childhood, puberty and early adulthood. While AMH may be of value in predicting reproductive lifespan (18, 19), it is of very limited value in predicting short-term fertility. Pregnancy in the presence of very low AMH level in post cancer patients has been reported (20), and

in healthy young women low AMH is not associated with reduced time to pregnancy (21).

Ovarian damage is not only related to patient factors such as age, but also to the therapeutic regimen to be administered. The majority of the chemotherapeutic drugs act against malignant cells by interfering with DNA replication, synthesis and repair, breaking double strands and disrupting the mitotic spindle. As such, treatment is based on the knowledge that cancer cells divide more rapidly than most other cell types. However, these same mechanisms of action are also cytotoxic for other tissues that have a physiologically high rate of cellular turnover. This leads to a plethora of side effects, one of which is on the reproductive system. Although factors such as drug class and dose, and the number of cycles of chemotherapeutic drugs administered, are strictly controlled, the effects that they can produce on human fertility are not easy to assess. Virtually all therapeutic regimens use drug combinations. As such, the role of individual drugs, as well as their synergistic effects, are difficult to assess, although some chemotherapeutic compounds have been classified as more gonadotoxic than others (22). Below, we review the drug class with the best understood and major effects on female reproduction, namely alkylating and alkylating-like drugs. Many more drugs are currently used in malignancy treatment, but unfortunately for most of them, lack of knowledge renders it difficult to assign the degree of ovotoxicity.

Alkylating and alkylating-like agents

Alkylating agents include some of the most cytotoxic compounds known and the ones mostly responsible for ovarian damage. Their major mechanism of action is to bind alkyl groups to DNA, with consequential damage that becomes particularly evident during subsequent cell replication. Because tumour cells have a high mitotic rate, they are less capable of repairing this damage than healthy cells, hence they are particularly likely to undergo cell death. The alkylating agent cyclophosphamide (CTX) is frequently used in the treatment of many solid and haematological tumours. Both human and animal studies have shown variable results, with different studies showing CTX damaging either the oocyte or the GCs of growing follicles (23-26). Significant damage has also been observed in the ovarian stromal tissue of patients treated

with alkylating agents, resulting in cortical blood vessel fibrosis (27). In a cohort study on 105 survivors examined on average 16 years after childhood cancer diagnosis, ovarian markers (ovarian volume and AMH level) were lower than those of control patients. Those markers, plus antral follicle count, were particularly affected in patients that had received very high doses of alkylating agents (CTX, ifosfamide and procarbazine). Significantly, only 8 of 105 patients had evidence of altered ovarian function (7). The CCSS also reported a significant correlation between increasing dose of alkylating agents and failure to achieve pregnancy (12).

Platinum-based agents such as cisplatin, carboplatin and oxaliplatin are considered to have alkylating-like properties, and are also essential compounds in many cancer therapies, including lung, breast, ovarian and colon cancers. They mainly act by binding to DNA and forming adducts which disrupt DNA transcription and replication and induce apoptosis (28). The effect on fertility caused by platinum-based therapy seems still unclear and is mainly considered as moderate (29, 30). A follow-up study performed over 15 years on malignant ovarian germ cell tumour patients who underwent fertility-sparing surgery showed that the probability of pregnancy significantly decreased as a consequence of cumulative doses of cisplatin-based therapy (31). Similarly, a German survey found a significant association between platinum-derived treatment and risk of infertility in female childhood cancer survivors in a study that followed patients up for around 20 years after treatment (32). Interestingly, in contrast with the other survey (12), Reinmuth et al. (32) did not find a correlation between CTX and infertility, but this is thought likely to be because they excluded patients from the study whose CTX-based therapeutic regimen was known to induce infertility, such as those with Hodgkin lymphoma. Several animal studies have confirmed that cisplatin can induce ovarian toxicity (33, 34). We have shown that exposure of mouse ovaries to cisplatin *in vitro* induces oocyte damage in early growing but not PFs: PF numbers are nonetheless drastically reduced, data that support the 'burnout' theory (35).

Therapeutic regimens for haematologic tumours such as Hodgkin lymphoma and of other cancers including brain tumours (36) require several cycles of aggressive chemotherapy using a drug

combination that often include procarbazine (PCZ). PCZ is metabolised *in vivo*, with its main metabolite inducing single-strand DNA breaks. Use of PCZ has frequently been associated with high rates of infertility (37), with one cohort study on 706 women treated for childhood cancer showing that the presence of PCZ in the therapeutic regimen increases the risk of early menopause by 150% for every gram of PCZ received by the patient (14). There is a study presently on going in childhood and adolescent Hodgkin lymphoma assessing whether replacing PCZ with dacarbazine might be equally effective but less toxic (38). A French follow-up study of childhood cancer survivors indicated that administration of melphalan alongside PCZ and CTX further increased the risk of POI (14). Melphalan is mainly used in the treatment of myeloma and retinoblastoma; its use seems to be related to better recovery of fertility when administered alone (39).

Sanders et al. (40) hypothesized that high dose of alkylating agents in bone marrow transplant patients increased the incidence of preterm delivery and low birth weight (LBW) of the newborns. More recently, there has been an indication that only the highest doses of alkylating agents might increase the risk of preterm birth (13), although the effect they found did not reach statistical significance, and LBW was only the consequence of early birth that their study indicated. If there is an effect on subsequent offspring, the cause of that effect is unclear, and could be also a consequence of stress in the mother-to-be survivor of the childhood cancer: certainly, stress effects such as this have been shown after several other kinds of emotional stressful conditions (13, 41).

Chemotherapy effects on the non-ovarian reproductive system

Radiotherapy is the major cause of damage caused by cancer treatment on reproductive tissues other than the ovary. This review, however, focuses solely on the role of cytotoxic drugs, for which, to the best of our knowledge, no data are currently available regarding their potential role on the hypothalamic-pituitary axis. Whether chemotherapeutic drugs can act on the central endocrine system causing hypopituitarism, pituitary atrophy or other hormonal disorders has

not yet been demonstrated (42). Clearly, though, effects such as this could cause detrimental consequences on fertility, and should be investigated. It is also well recognised that radiotherapy impacts on uterus development and functionality (40, 43) but data on the effects of chemotherapy on clinical uterine function (i.e. pregnancy outcome) are largely very reassuring (12, 44). Uteri of patients who received chemotherapy treatment during childhood were however more likely to be of smaller volume with poorer vascularization than age-matched healthy controls, even with normal ovarian function (45, 46). Both uterine size and blood flow have been associated with reduced outcome of assisted reproduction treatment (47), but the above data do not, overall, indicate a major risk for adverse obstetric outcome.

Effect of chemotherapy when administered during pregnancy

The concurrence of cancer and pregnancy is a rare event, occurring only in around 0.1% of all pregnancies, although the increasing age at childbearing will likely produce an increase in its incidence (48). In past years, abortion was routinely recommended by oncologists, both for the possibility that the therapy could damage the fetus and in order not to compromise the mother's survival. In the majority of cases, both concerns have proven unfounded. When chemotherapy is administered to pregnant women after the first trimester, there is no evidence of higher malformations rate, longer term deficiencies or health problems in the resulting offspring (49-52). In addition, with the exception of highly aggressive and systemic tumours that require immediate treatment, there is no evidence that pregnancy interruption will improve the mother's survival (53).

The ability of chemotherapeutic drugs to cross the placental barrier varies from drug to drug, as does the degree of cytotoxic damage that they can produce, with antimetabolites and alkylating agents thought to be the most teratogenic. However, fetal damage is also affected by the developmental stage, with more frequent malformations occurring when cancer therapy is administered during organogenesis (48). There is also evidence from a mouse model that in utero exposure to alkylating agents could adversely

affect the developing gonads: CTX exposure during early pregnancy reduced the number of PFs in the offspring ovaries and increased follicle activation, which in turn would lead to a further faster deprivation of the ovarian reserve (54). This study, if confirmed in women, would open new concerns when exposing pregnant women to chemotherapy.

A further, indirect, effect of cancer treatment on fertility is the need to postpone pregnancy after treatment, generally until at least 6 months and often 2 years after the end of chemotherapy (5). Survivors of hormone-sensitive breast cancer are often prescribed tamoxifen therapy for 5 -10 years, further delaying the possibility of subsequent motherhood: this duration of delay will have a significant impact on fertility for many adult women. However, several studies have failed to find an increase of reoccurrence/relapse or of lower subsequent life expectancy in women who become pregnant after breast cancer (54, 55), thus this is a complex subject for discussion between a woman and her oncologist.

Surprisingly, a report from the American CCSS has shown that cancer survivors are slightly more likely than their female siblings to have induced abortion (12), while another American survey demonstrated that cancer survivors have a higher risk of undesired pregnancy than the average population, with that risk increasing with age. Both of these are thought likely to be due either to patients underestimating their fertility after cancer treatment (56) or to concerns about their and their offspring's health (57). A similar Danish cohort study also showed a small increase in the proportion of survivors who opted for abortion in comparison with their sisters (58).

Overall, for those cancer survivors who are likely to remain fertile, contraception counselling is an important aspect of care (59).

Future directions for fertility damage assessment

Large follow-up studies are gradually increasing our knowledge of the long term effects of chemotherapy on fertility in children, young girls and women. However, these data require long periods of time to be gathered and analysed. The vital information obtained by these

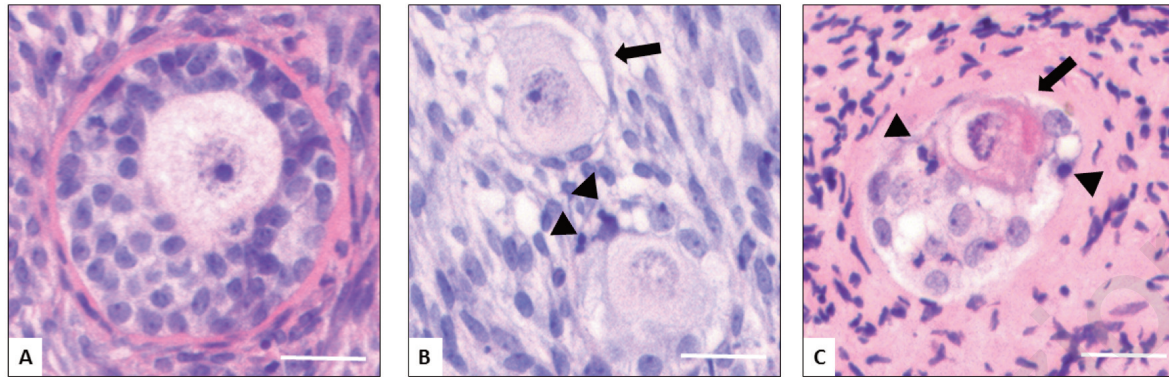


Figure 2 - Representative microphotographs of human ovarian follicles at preantral stage. Human ovarian cortical strips were cultured for 6 days in: (A) drug-free medium (control), or in the presence of (B) 5 µg/ml cisplatin and (C) 2 µg/ml doxorubicin during Day 2 of culture. Follicles in B and C display signs of GCs pyknosis (head arrows) and oocyte damage (arrows). Scale bars: 25µm. Unpublished data: F Lopes, S Morgan, RA Anderson and N Spears.

studies should allow further development of therapeutic regimens. However, these clinical studies may not provide clear evidence about the specific role of any individual drug. Instead, such knowledge is likely to require either animal studies, *ex vivo* human tissue work, or a combination of the two. A xenografting mouse model has been developed using fetal human ovaries to test drug damage, producing data consistent with CTX ovotoxicity (60). Although this is a valuable method to directly assess the ovarian reserve, it is not without issues, such as the paucity of experimental human material as well as the on going mitotic division in the fetal germ cell population of the mid-trimester ovary that was used, not fully representative of the resting pool of PFs. Ovarian tissues collected from young or adult women overcome that problem. Soleimani et al. (61) tested the effect of doxorubicin (DOX) on human ovarian cortex collected from patients undergoing preventive ovarian cryopreservation, using *in vitro* and *in vivo* in xenografted mice methods. This showed that DOX induces apoptosis of primordial and growing follicles, adversely affecting both oocytes and GCs, as well as damaging stromal vasculature. Our laboratory is undertaking similar investigations using an *in vitro* approach to test the effect of chemotherapy drugs on adult human ovarian cortex. Ovarian cortical strips collected from consented women undergoing elective Caesarean section have been used to test the effects of two chemotherapy drugs for which there is debate about their ovotoxicity, namely cisplatin and DOX, with results indicating that both drugs damage early growing ovarian follicles (Figure 2).

Conclusion

For the majority of women, there is an innate desire to have children. Infertility can be an unwanted consequence of life-saving cancer treatment, and may result in significant emotional distress, leading to reduced quality of life. Moreover, even in cancer survivors in whom fertility is preserved, some women experience anxiety about their potential future reproductive capability. Such concerns include fears that the toxic therapy received could potentially affect their progeny; that pregnancy could result in a relapse/recurrence; or that their shorter life expectancy could lead to a failure to provide adequate childcare. Iatrogenic infertility also negatively impacts on physical well-being. The premature loss of the ovarian reserve as a side effect of some chemotherapeutic drug treatments may lead to an increased rate of cardiovascular disease and osteoporosis, as well as to increased mortality rates. Due to all these factors, preserving the reproductive function of cancer survivors is of utmost importance. The accumulating knowledge about the role of chemotherapy on fertility and pregnancy outcome makes it possible to reassure many women about their childbearing potential after cancer treatment, as well as identifying those patients with higher risk of infertility. Together, early fertility counselling, investigation of ovarian reserve, the use of cancer therapies with reduced effect on fertility and fertility preservation techniques (reviewed in 62) combine to support the maternal aspirations of many cancer survivors at risk of reproductive impairment.

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