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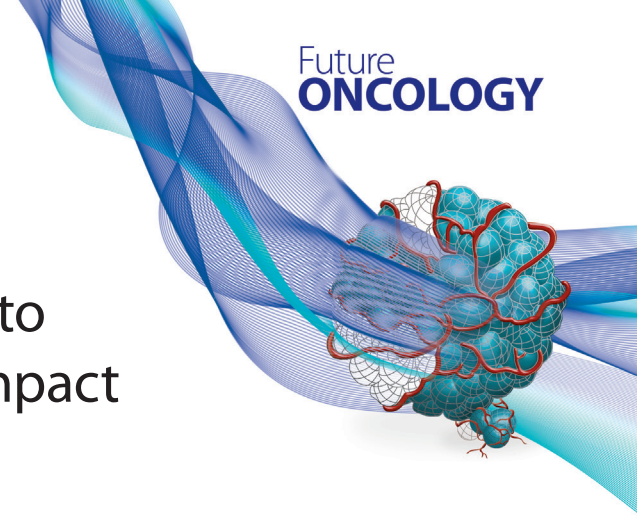

Future ONCOLOGY

Chemotherapy-induced damage to ovary: mechanisms and clinical impact

Giuliano Bedoschi[1,2,3], Paula Andrea Navarro[3] & Kutluk Oktay*[,1,2]

Cancer is a major public health problem around the world. Currently, about 5% of women diagnosed with cancer are of reproductive age. These young survivors may face compromised fertility. The effects of chemotherapeutic agents on ovarian reserve and its clinical consequences are generally inferred from a variety of surrogate markers of ovarian reserve, all aiming to provide prognostic information on fertility or the likelihood of success of infertility treatment. Until recently, the mechanisms that are responsible for chemotherapy-induced ovarian damage were not fully elucidated. The understanding of these mechanisms may lead to targeted treatments to preserve fertility. In this manuscript, we will review the current knowledge on the mechanism of ovarian damage and clinical impact of chemotherapy agents on fertility.

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KEYWORDS
• anti-Mullerian hormone • antral follicle count • chemotherapy • fertility preservation • follicle-stimulating hormone • ovarian aging • ovarian damage • ovarian reserve

Cancer is a major public health problem around the world and is the second leading cause of death in the USA [1]. In 2016, approximately 844,000 new cases of cancer will be diagnosed in women in the USA [1]. In recent years, the remarkable screening, diagnostic and therapeutic advances in oncology practice improved the prognosis for many cancer patients, adding years to their anticipated survival. In fact, all these measures have resulted in a 23% drop in the cancer death rates from 1991 to 2012 [1].

Currently, about 5% of cancers affects women younger than 50 years [1]. As young patients with these once-fatal malignancies become long-term survivors, many must face the potentially devastating complications of the treatment. Young survivors will likely face compromised fertility that is now recognized as among the most prevalent long-term side effects of cancer therapy. The prospect of partial or total infertility can significantly add to anxiety and emotional strain during disease management, and may also compromise quality of life [2]. To offset these risks, women can be offered several options for fertility preservation, including conservative cancer management, and cryopreservation of oocyte, embryo or ovarian tissue. Embryo and oocyte cryopreservation are considered established fertility preservation techniques and have been widely applied across the world [3,4]. On the other hand, ovarian tissue cryopreservation is still considered an experimental technique, despite advances in recent years [5].

Studies exploring the mechanisms behind the actions of the different chemotherapy agents are providing greater information as to the specific effects of each agent on the different cell types of the ovary. The effects of antineoplastic agents on the ovaries are clinically inferred from a variety

[1]Innovation Institute for Fertility Preservation & *In vitro* Fertilization, New York, NY, USA
[2]Laboratory of Molecular Reproduction & Fertility Preservation, Obstetrics & Gynecology, New York Medical College, Valhalla, NY, USA
[3]Division of Reproductive Medicine, Department of Gynecology & Obstetrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
*Author for correspondence: koktay@fertilitypreservation.org

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of surrogate markers, including the incidence of amenorrhea, serum measurements of early phase follicle-stimulating hormone (FSH) and estradiol (E2) concentrations, antral follicle count (AFC) determined by transvaginal ultrasound and anti-Mullerian hormone (AMH) serum concentrations.

This article will review all the mechanism and clinical impact of chemotherapy on ovarian reserve.

Ovarian aging

The ovary has a finite endowment of primordial follicles that is established during the second half of intrauterine life, followed by a steady decline until menopause. Each primordial follicle consists of an immature oocyte surrounded by a single layer of granulosa cells. The primordial follicles constitute the ovarian reserve and are continuously recruited throughout life accounting for a progressive declining number of follicles. Once activated follicular growth, both oocyte and granulosa cells begin sequential stages of growth and development. Therefore, reproductive age women have follicles in different stages of development in the ovaries due to the continued recruitment of primordial follicles. Most recruited primordial follicles undergo atresia and only a minority of the follicles reaches the preovulatory stage. The follicle atresia occurs in response to unfavorable changes in many factors, such as follicles response to gonadotropins, autocrine and paracrine factors. Apoptosis, or programmed cell death, occurs as a result of these adverse changes.

The loss of primordial follicles occurs continuously throughout the years, reducing pregnancy rates in a progressive manner, until the end of ovarian reserve by about 50 years. The number of primordial follicles recruited in each menstrual cycle appears to be a fixed proportion of the remaining primordial follicles in the ovary, with an increased recruitment in young women. Other factors than age may also contribute to follicle atresia, such as smoking, stress, parity and body mass index [6]. Although recently challenged [7], most of the scientific evidence indicates that the number of primordial follicles constituting ovarian reserve is finite.

Mechanisms of chemotherapy-induced ovarian damage

The chemotherapeutic agents act by different mechanisms in the ovary. This has clear relevance to the understanding of which aspects of ovarian function are most affected by these agents. Within the ovarian follicle, both oocyte and granulosa cells are vulnerable to damage caused by chemotherapy. Each class of chemotherapeutic agent may have different mechanism of action on cancer cells, with the end result being halt the cell division cycle **(Table 1)**.

• Assessing the impact of chemotherapy on ovarian reserve by histological analysis

Previous histological studies in human ovaries have shown that chemotherapy treatments can cause loss of primordial follicles and ovarian atrophy [9,10]. A study by our group had also assessed the impact of chemotherapy on ovarian reserve by histological analysis [11]. Samples from 26 patients who underwent ovarian tissue cryopreservation for fertility preservation were evaluated histologically. Out of the 26 patients, ten had received prior chemotherapy while 16 did not (control group). Patients who received chemotherapy had similar average age and significantly lower primordial follicle counts than the control group. In addition, patients treated with alkylating regimens had significantly lower counts of primordial follicles when compared with those receiving non-alkylating agents or to age-controlled women that have not received any chemotherapy. This quantitative study showed that chemotherapy regimens, especially those containing alkylating agents, result in significant loss of ovarian reserve **(Figure 1)**.

• Apoptotic death of human primordial follicle

The oocyte death by apoptosis was identified as the main mechanism responsible for loss of germ cells and premature ovarian failure. As one cannot ethically study *in vivo* impact of chemo on ovarian follicles in patients, researchers studied this question in rodent studies [12–14] and in models of human ovarian xenograft [8,15]. These well-established xenograft models clearly showed that chemo-induced primordial follicle death is apoptotic in human ovary.

Previous studies in our laboratory had shown that several chemotherapeutic agents alter fertility in reproductive-age models. We have previously developed a xenograft model to characterize the *in vivo* impact of cyclophosphamide in the human ovary [15]. In that human ovarian xenograft model, a single dose

Table 1. Classes of chemotherapy, their action and infertility risk.

Class of agent	Examples	Mechanism of action	Infertility risk
Alkylating agents	Cyclophosphamide Mechlorethamine Chlorambucil Busulfan Melphalan	The active metabolites form cross-links with DNA with resultant inhibition of DNA synthesis and function. DNA double strand breaks and resultant P63-mediated apoptotic death in human primordial follicles [8]	High risk
Platinum-based compounds	Cisplatin Carboplatin	Covalently binds to DNA to form intra- and interstrand DNA cross-links, leading to DNA breakage during replication. This inhibits DNA transcription, synthesis and function. Specific toxicity has not been shown in human primordial follicles	Intermediate risk
Antimetabolites	Methotrexate 5-fluorouracil Cytarabine	Inhibition of DNA, RNA, thymidylate and purine synthesis. No DNA damage in human follicles, hence not gonadotoxic	Low risk
Vinca alkaloids	Vincristine Vinblastine	Inhibition of tubulin polymerization and disruption of microtubule assembly during mitosis. This arrests mitosis during metaphase and leads to cell death. No DNA damage in human follicles, hence not gonadotoxic	Low risk
Anthracyclin antibiotics	Daunorubicin Bleomycin Adriamycin (doxorubicin)	Inhibition of DNA synthesis and function. It interferes with DAN transcription. It inhibits topoisomerase II, which leads to DNA breaks. It also forms toxic oxygen-free radicals, which induce DNA strand breaks, thereby inhibiting DNA synthesis and function. Doxorubicin induces DNA double strand breaks P63-mediated apoptotic death in human primordial follicles [8]	Low risk (except adriamycin: intermediate risk)

of cyclophosphamide resulted in significant primordial follicle death by apoptosis. The grafts were recovered after 12–72 h after the injection and were sectioned for AFC. Although the peak of primordial follicle loss occurred 48 h after cyclophosphamide injection, the molecular evidence for apoptosis, as evaluated by terminal nucleotidyl transferase-mediated nick end labeling (TUNEL) assay, peaked much earlier at 12 h after the injection of cyclophosphamide, indicating that damage to primordial follicles is initiated almost immediately upon exposure to cyclophosphamide.

In subsequent studies, we further investigated the mechanism of chemotherapy-induced oocyte apoptosis. Chemotherapy agents act causing damage to the DNA of oocyte cells. Among the various types of DNA damage, double-stranded DNA breaks are the most detrimental type of damage. The oocyte initially attempts to repair the DNA damage through the ataxia-telangiectasia mutated (ATM)-mediated DNA damage repair pathway. For cells in which the DNA damage cannot be repaired, elimination occurs via apoptosis, unless the cell is arrested in growth (cell senescence) **(Figure 2)**. We showed in *in vitro* and human ovarian xenograft models that doxorubicin [8] and cyclophosphamide [16] induce DNA double-stranded DNA breaks in primordial follicles, which trigger the apoptotic process and death in most cases. Interestingly, we have also shown that the ATM-mediated pathway may be activated in response to this damage and may repair and rescue some primordial follicles in the face of chemotherapy-induced genomic insult [17]. Hence research from our laboratory suggests that alkylating agents and those in the topoisomerase inhibitor category (e.g., doxorubicin) are damaging to primordial follicle oocytes and produce permanent damage to ovarian reserve though some follicles may be able to survive due to their ability to repair DNA damage.

In addition, as mitotic cells are sensitive to any class of chemotherapeutic agents, follicle development is altered in growing follicles, explaining the temporary amenorrhea and declining AMH levels that may be seen even with chemotherapeutic agents that are known to have low toxicity on primordial follicle oocytes [8,15].

• Impact of chemotherapy on stromal/vascular function in ovary

Histological studies also indicate indirect effects of chemotherapy damage by stromal cells [18]. Chemotherapeutic agents are associated with a variety of heterogeneous vascular complications [19]. *In vivo* monitoring of blood flow after the administration of doxorubicin showed a sharp reduction in ovarian blood volume and spasm of small vessels in the ovary [20]. In addition, damage to blood vessels and focal fibrosis of the ovarian cortex are other mechanisms involved in the chemotherapy-induced ovarian damage.

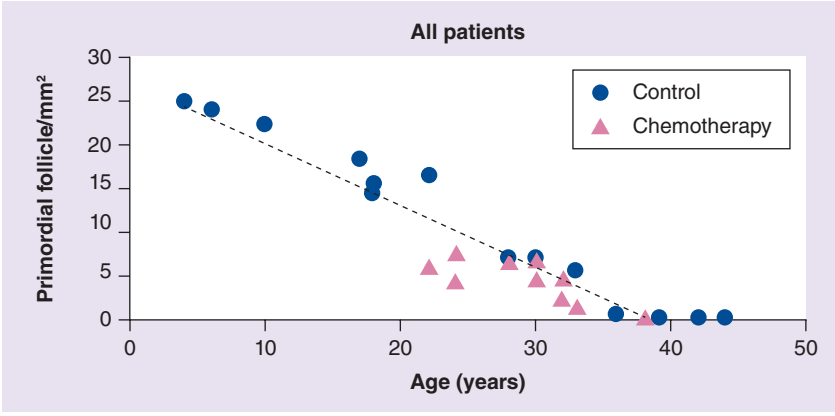

Figure 1. Quantitative assessment of the impact of chemotherapy and age on primordial follicle reserve. While age is a significant determinant of ovarian reserve, chemotherapy administration accelerated the age-related decline in follicle reserve compared with controls. Based on this graph, a typical gonadotoxic chemotherapy protocol results in the loss of 10 years' worth of ovarian reserve. Published with permission from [11].

In a previous study by our laboratory [8], human ovarian tissues were cultured in multiwell plates with doxorubicin or sterile normal saline. After 24–72 h of culture, the tissues were evaluated for vascular density and neoangeogenesis by immunohistochemistry. This study showed that doxorubicin causes a decrease in new blood vessels density in a dose-dependent manner when compared with control. In contrast, new blood vessel density increased significantly after 72 h culture in controls when compared with the uncultured baseline tissue, due to expected continuation of microvessel proliferation. Doxorubicin also caused a significant decrease in the density of mature blood vessels in a dose-dependent manner when compared with controls. Validating and confirming *in vitro* organ culture experiments, doxorubicin treatment also resulted in reduced vascular density in xenografted human ovarian tissues compared with controls. These results are consistent with other studies that have demonstrated that chemotherapy may induce stromal fibrosis and ovarian vascular abnormalities [18,21].

While it is challenging to demonstrate that vascular damage directly contributes to chemotherapy-induced ovarian damage, in a recent study we showed an inverse correlation between ovarian vascular density and primordial follicles apoptosis [22]. It is normally supposed that primordial follicles do not depend on blood perfusion, but clinical experience from ovarian tissue transplantation do not support this belief [5]. While growing follicles are more sensitive to acute ischemic changes due to the presence of a larger amount of granulosa cells, our experimental models clearly support that primordial follicles depend on adequate vascularization [22]. Owing to this, it is possible that ovarian vascular injury may be an indirect mechanism by which chemotherapy reduces the number of primordial follicles.

It is worth to mention that the chemotherapeutic agents may damage the endocrine ovarian function, both in premenopausal and postmenopausal women, impairing the production of sex steroids such as testosterone and estrogen [23].

• Indirect damage to primordial follicles: increased follicle activation

A recent study has proposed a new hypothesis to the chemotherapy-induced ovarian damage, suggesting that chemotherapy causes an increase in follicular recruitment, causing depletion of ovarian reserve and consequently ovarian failure [24]. The injury to the growing follicles reduces its inhibitory effects on primordial follicles recruitment, thus resulting in activation of the primordial follicles in an attempt to replace the cohort of damaged antral/preovulatory follicles [25]. However, the lack of explanation of the age-related differences in sensitivity to chemotherapy-induced damage to ovary, the use of nonspecific index of follicular activation rate and the uncertainty in determining the follicular apoptosis source (oocyte vs granulosa cells, or both), are among the major limitations of this study. Furthermore, this study was limited to rodent data and several other studies utilizing rodents did show presence of apoptosis in oocytes of primordial follicles after chemotherapy [26,27]. One of the other major weaknesses of the follicle activation theory is that it cannot account for the increased abnormalities seen in offspring of mice, which were exposed to chemotherapy immediately before conception [28]. If the main cause of follicular depletion was due to follicular activation, these effects or late transgenerational defects would not be expected. This increase in anomalies, however, is highly consistent with the findings of primordial follicle oocyte DNA damage as shown by our group. Likewise, claims for treatments to prevent chemotherapy-induced ovarian follicle loss by suppressing this presumed activation is unfounded given that, it would in theory, preserve DNA-damaged primordial follicle oocytes that have been shown to result in

abnormal pregnancies by the same group which put forward the follicle activation theory [29].

Given this information, we believe that the data are insufficient to support chemotherapy-induced activation as a material cause of follicle loss, if any, and recent published and unpublished data have supported the same contention. In contrast, the evidence for DNA damage induced follicle death is highly convincing and this DNA damage-induced oocyte death appears to be mediated by a germ cell specific p53 family member transcription factor, TAp63 [8,27]. Other DNA damaging treatments such as radiation have been shown to activate TAP63-α and result in primordial follicle apoptosis *in vivo* in rodents [30].

Impact of chemotherapeutic agents on ovarian reserve

Clinically, the impact of chemotherapy treatments in the ovaries range from none, throughout different levels of partial damage resulting in reduced fertility, until the full damage with total loss of primordial follicles, ovarian atrophy and complete ovarian failure. The degree of ovarian damage and risk of infertility depends on the dose and type of chemotherapeutic agent, and is related to the woman's age at the time of treatment, with greater risk of infertility and ovarian failure in patients with advanced reproductive age [31,32]. This is primarily due to reduction in ovarian reserve that occurs naturally with age, since older women have less primordial

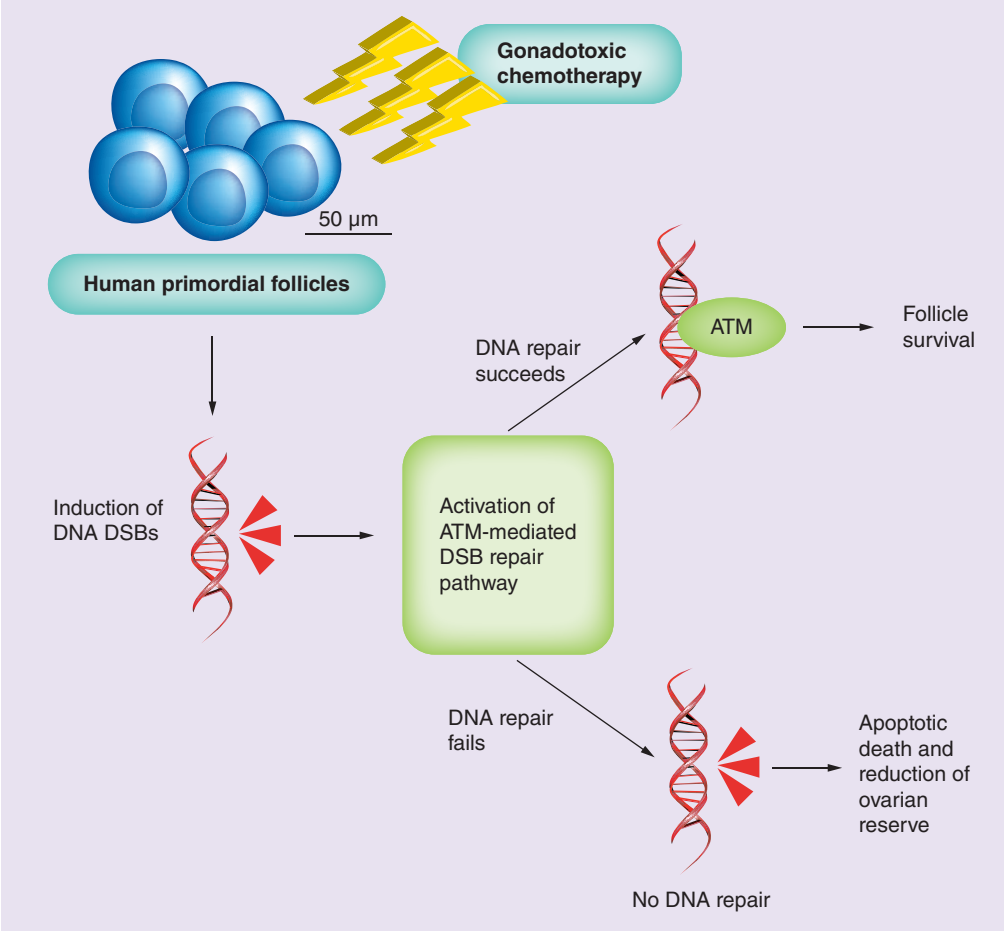

Figure 2. The mechanism of chemotherapy-induced damage to ovary. The administration of chemotherapy agents such as cyclophosphamide and doxorubicin results in the DNA DSBs in primordial follicle oocytes. This damage activates the ATM-mediated DNA repair pathway. Oocytes with sufficient DNA repair ability may survive this genotoxic stress while others with less efficient repair may be lost as a result of severe DNA damage triggering apoptotic death pathways or cell senescence.
ATM: Ataxia-telangiectasia mutated; DSB: Double strand break.

follicles, as mentioned above. The decline in the number of follicles after chemotherapy can lead to decreased reproductive potential, ovarian insufficiency and menopause many years after the oncological treatment, even in patients undergoing chemotherapy during infancy [33].

Several studies use the amenorrhea rate (both acute and chronic) as a marker of ovarian failure and/or risk of infertility in patients undergoing chemotherapy. However, the presence or absence of menstruation is an inaccurate assessment of ovarian function. It is worth mentioning that the loss of conception potential occurs about 10 years before menopause/ovarian failure in healthy women [34]. Meanwhile, women might have regular menstrual cycles for several years after chemotherapy, but may have lower likelihood of pregnancy during this period due to a significant reduction in ovarian reserve. It would be remarkably useful to consider the utility of ovarian reserve markers (biochemical and/or ultrasonographic) to allow detection of varying levels of ovarian reserve damage before ovarian failure [35]. This would allow improved analysis of the effects of chemotherapeutic agents in the ovarian reserve and would allow individualized counseling based on pre- and post-chemotherapy analysis of the ovarian reserve.

Markers of ovarian reserve

The effects of chemotherapeutic agents on ovarian reserve and its clinical consequences are generally inferred from a variety of surrogate markers of ovarian reserve, all aiming to predict fertility or provide prognostic information on the likelihood of success of assisted reproduction treatment. Ovarian reserve tests include biochemical and ultrasonographic markers. **Table 2** lists the available ovarian reserve markers.

• Basal FSH & E2

The ovarian function depends on gonadotropin production by the pituitary gland. FSH stimulates the growth of granulosa cells of growing follicles as well as stimulates the production of estradiol by the follicles. Since the serum FSH concentrations vary significantly throughout the menstrual cycle, the value of serum FSH is best obtained during the early follicular phase. It is worth to mention that the FSH levels can vary significantly between different menstrual cycles. Since elevated levels of FSH are one of the earliest indications of reproductive age in women, its use as an ovarian reserve test is widely applied in clinical practice. In women with ovarian reserve compromised by chemotherapy, follicular depletion correlates to an increase in FSH levels. Several studies have evaluated the relationship between levels of FSH and *in vitro* fertilization (IVF) cycles outcomes. The FSH levels correlate to ovarian stimulation response and to a lesser extent, with the likelihood of successful treatment [36,37]. Furthermore, higher levels of FSH have high specificity to predict poor response to ovarian stimulation, but their sensitivity to identify these women is generally low and decreases with the value of the used threshold [35]. In addition, high FSH concentrations in young women presenting amenorrhea may suggest premature ovarian failure. Estradiol serum concentration during the early follicular phase, itself, has little value as testing ovarian reserve, but may provide additional information to the interpretation of basal FSH level, especially when FSH levels are within the normal range [35].

• Inhibin-B

Inhibin-B is a glycoprotein hormone mainly secreted during the follicular phase by granulosa cells of preantral and antral follicles [38]. Inhibin-B controls the pituitary FSH secretion by negative feedback mechanisms. As inhibin-B levels decrease with advancing reproductive age and decreased ovarian reserve, FSH levels increase [39]. However, inhibin-B levels vary widely during and between menstrual cycles and have low sensitivity and specificity in predicting ovarian response in ovarian stimulation [40].

• Anti-Mullerian hormone

AMH is a glycoprotein hormone produced by granulosa cells from primary, preantral and small antral follicles and is involved in regulating primordial follicle recruitment [41,42]. Small antral follicles tend to be the main source of AMH because they contain larger numbers of granulosa cells. The number of small antral follicles is correlated with the number of primordial follicles and AMH levels decline progressively with age, becoming undetectable by menopause [43]. Because small antral follicles secrete AMH in a gonadotropin-independent state, they exhibit little variation within and between menstrual cycles [44]. This can be extremely important in women diagnosed with cancer who may have urgency to test for ovarian reserve before cancer treatment, as these women may not have to wait for the beginning of the next menstrual cycle.

Table 2. Ovarian reserve markers used to identify diminished ovarian reserve.

Ovarian reserve markers	Diminished ovarian reserve
Early follicular phase serum follicle stimulation hormone level	FSH levels greater than 10 IU/l on menstrual cycle day 2 or 3 have high specificity, but low sensitivity for predicting low ovarian reserve
Early follicular phase serum inhibin-B level	Inhibin-B levels are generally lower in women with diminished ovarian reserve. It is not considered as a reliable measure of ovarian reserve because of production from larger follicles and technical challenges with the assay
Serum AMH level	Low AMH threshold values have good sensitivity and specificity for low ovarian reserve. The technique of AMH assay can result in variation in results
Ovarian volume	Low ovarian volume has high specificity and low sensibility for predicting low ovarian reserve and has limited clinical utility as an ovarian reserve marker
Total AFC	Low AFC on cycle days 2–3 of menstrual cycle has high specificity for predicting low ovarian reserve. The accuracy is operator dependent

AFC: Antral follicle count; AMH: Anti-Mullerian hormone; FSH: Follicle-stimulating hormone.

In addition, the AMH is a very promising marker for women who have received chemotherapy, as it is an important test to identify women with low ovarian reserve and has good specificity for poor ovarian response, and to a lesser extent, for pregnancy [45]. Although AMH level is a good predictor of oocyte quantity, it may not provide information about oocyte quality. Thus, young survivors with low AMH levels may have a reduced number of oocytes but normal, age-appropriate oocyte quality [46]. With further research, AMH level testing may become increasingly valuable in assessing ovarian reserve for reproductive age women diagnosed with cancer [47]. Measuring AMH levels before and after chemotherapy allows detection of differences in ovarian damage among chemotherapy regimens and may help assess long-term ovarian function [47,48].

It is worth to mention that there have also been some technical limitations for AMH. First, there are different existent forms of the assay. Consequently, when applying results in clinical practice, it is important to know which assay method was used to measure AMH. Second, the sample handling can dramatically alter AMH concentrations. Clear guidance on how samples should be collected, processed and stored may avoid sample instability and lack of reliable interassay comparability [49].

• Antral follicle count

The AFC records the total number of antral follicles measuring 2–10 mm on both ovaries that are observed during transvaginal ultrasound in the early follicular phase [50]. The number of antral follicles correlates with the number of remaining primordial follicles, and good intercycle and interobserver reliability has been demonstrated [51]. For this reason, as the supply of primordial follicles decreases, the number of antral follicles visible on ultrasound also declines [50]. A low AFC is associated with poor response to ovarian stimulation during IVF, but it does not reliably predict failure to conceive.

Clinical detection of ovarian damage

Several studies have evaluated the use of ovarian reserve markers for detecting compromised ovarian reserve in children [52–54] and adults [55,56] diagnosed with cancer that received chemotherapy. Data from prospective studies are still limited, but also illustrate the ovarian damage after chemotherapy.

• Age & impaired ovarian reserve

Age and type/dose of chemotherapy are important risk factors for ovarian failure, with older women having higher risk than younger women [57]. Some studies have clearly demonstrated the relationship between age and the impact of ovarian reserve. Petrek *et al.* found a higher incidence of amenorrhea after chemotherapy in women aged more than 40 years diagnosed with breast cancer when compared with younger women [58]. Gracia *et al.* conducted a cross-sectional analysis of a prospective cohort study to determine whether ovarian reserve markers differ among women of reproductive age exposed to chemotherapy compared with healthy women of the same age or with healthy women of advanced reproductive age. Ovarian reserve markers were compromised in the study group when compared with age-matched

controls. Ovarian reserve markers in the study group were similar to those presented by the group of women with advanced reproductive age [59]. A recent study showed that women diagnosed with breast cancer over 32 years old have higher rates of amenorrhea after chemotherapy with alkylating agents when compared with younger women. In addition, these patients presented compromised ovarian reserve markers, demonstrating impact of chemotherapy on fertility [60].

• Biochemical markers & impaired ovarian reserve

Early follicular phase serum FSH is not a good marker of ovarian reserve in women who underwent cancer treatment. Women presenting FSH levels within the normal range may have suffered significant damage to the ovaries. Studies have shown that FSH levels were significantly higher in women presenting amenorrhea after oncological treatment, however the FSH levels were within the normal range in women with diminished ovarian reserve but regular menstrual cycles [61,62]. Women with diminished ovarian reserve have a limited opportunity window to conceive naturally because they present higher risks of ovarian failure and infertility. Therefore, it would be useful to identify these women as early as possible, so they are properly counseled about future chances of pregnancy.

Some case–control studies evaluating patients diagnosed with early breast cancer, hematological cancer and childhood cancer have shown that AMH levels are significantly lower after chemotherapy when compared with age-matched control group of healthy women [53,63–64].

Several studies have explored the serial changes in the AMH levels in women before and after chemotherapy. Anders *et al.* demonstrated that breast cancer patients with baseline AMH less than 1.09 ng/ml had increased risk of amenorrhea after chemotherapy [65]. Anderson *et al.* performed a similar study and found baseline AMH less than 1.9 ng/ml as a predictor of risk for amenorrhea in women diagnosed with breast cancer [66]. According to these studies, baseline AMH is useful for predicting the risk of amenorrhea after chemotherapy. A recent study found baseline AMH less than 1.87 ng/ml as a predictor of risk for the occurrence of amenorrhea in women diagnosed with breast cancer undergoing chemotherapy [60]. However, given that amenorrhea means the presence of irreversible damage to the ovaries, the authors of this study indicated the need for fertility preservation counseling in women of reproductive age with baseline AMH less than 3.32 ng/ml, as these women may have compromised fertility after chemotherapy, without necessarily presenting amenorrhea [60]. Similar to the findings of these studies, other prospective studies evaluated women diagnosed with hematological cancer, demonstrating that AMH levels after chemotherapy were significantly lower than baseline AMH levels [55,67]. In addition, the basal AMH levels in patients diagnosed with hematological cancer were significantly lower than those found in age-matched control group of healthy women. Further studies evaluating this specific group of patients are necessary [55,68].

The basal AMH levels in the setting of patients diagnosed with cancer undergoing fertility preservation treatment were also evaluated. AMH levels were analyzed in 126 reproductive-age women diagnosed with early breast cancer who underwent oocyte or embryos cryopreservation. The mean levels were 2.5 ± 2.3 ng/ml (mean ± standard deviation). None of the 23 patients with AMH greater than 1.2 ng/ml presented low response to ovarian stimulation. A significant proportion of patients with AMH less than 1.2 ng/ml (seven of 18 patients) presented a poor response to ovarian stimulation [69].

• Ultrasonographic markers & impaired ovarian reserve

Regarding the AFC, a recent study demonstrated that women of reproductive age diagnosed with breast cancer showing less than 13 antral follicles in total, regardless of age, are at increased risk of developing amenorrhea after cancer treatment [60]. Anderson *et al.* evaluated 56 reproductive-age women diagnosed with breast cancer. The authors performed AFC before the initiation of chemotherapy and found a higher AFC in women who remained ovulatory after chemotherapy when compared with women who developed amenorrhea (19 vs eight follicles) [66].

Future perspective

Understanding of the risks of infertility in women treated with chemotherapy has advanced in the last years. Clinical studies evaluating the chemotherapy-related ovarian damage, employing surrogate ovarian reserve markers, may improve healthcare providers' knowledge about infertility as a potential risk of oncological therapy and may enable them to recommend

fertility preservation techniques in women at risk. Fertility counseling should be patient-tailored, since both the impact of chemotherapy on ovarian reserve and the success of fertility preservation techniques are strongly linked to patients' age, ovarian reserve and type and dose of chemotherapy regimen.

Advances in our understanding of the mechanisms involved in chemotherapy-induced damage to ovarian reserve have opened new prospects for fertility preservation treatments. These advances occurred mainly in the investigation of pharmacological agents to protect ovarian reserve during chemotherapy. Cytotoxic agents have different mechanisms of damage to the various cell populations within the ovaries, providing different targets for potential attenuating agents [70,71]. Most of these protective agents are in preliminary stages of study and future developments in these areas will depend on accurate evaluation of the effictiveness of each potential pharmacological agent. Furthermore, there is a significant need to demonstrate that co-treatment with these agents does not interfere with the efficacy of cancer treatment, or produce genetically compromised embryos.

EXECUTIVE SUMMARY

Background

- Gonadotoxic chemotherapy often results in premature ovarian failure and infertility.
- The age of the patient, the type and dose of chemotherapy are the main factors determining the magnitude of the damage in the ovary.
- Preservation of ovarian function and fertility has become one of the major quality of life issues for patients undergoing chemotherapy at reproductive age.

Mechanisms of ovarian damage

- The ovaries are adversely affected by chemotherapy regimens. Accelerated and premature depletion of germ cells in the gonads caused by direct toxic insults to the primordial follicle oocyte is the main mechanism underlying gonadal failure. In addition, damage to ovarian stroma and microvascular architecture may be contributory to this damage.

Clinical impact of chemotherapy

- Ovarian reserve tests may help to predict a woman's reproductive lifespan and may be useful for individualization of fertility preservation strategies before gonadotoxic chemotherapy, as well as for detecting loss of ovarian reserve in patients who received chemotherapy.

Ovarian reserve markers

- Anti-Mullerian hormone (AMH): AMH is produced by granulosa cells from preantral and small antral follicles and is involved in regulating primordial follicle recruitment. AMH level represents the ovarian follicular pool and is a useful marker of ovarian reserve.
- Antral follicle count (AFC): The AFC is considered a reliable noninvasive method for determining the ovarian reserve, as the number of antral follicles is proportional to the number of nongrowing follicles that are remaining in the ovary. AFC is measured by transvaginal ultrasonography in the early follicular phase. The numbers of follicles in both ovaries are added for the total AFC.
- Follicle-stimulating hormone (FSH): Early follicular phase (basal) FSH is the most used test in determining ovarian reserve. FSH concentrations vary significantly throughout the menstrual cycle and the value of serum FSH is best obtained during the early follicular phase. FSH levels can vary significantly between different menstrual cycles.
- Estradiol: Basal estradiol levels may provide additional useful information for the evaluation of ovarian reserve, especially when FSH levels are within the normal range.

Future perspective

- Fertility counseling should be patient-tailored, since both the impact of chemotherapy on ovarian reserve and the success of fertility preservation techniques are strongly linked to patients' age, ovarian reserve and type and dose of chemotherapy regimen.
- The study of new pharmacological agents for fertility preservation during chemotherapy should consider both treatment effectiveness and safety.

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