Cristiana Lopes Martinho

Effects of chemotherapy on ovarian reserve and fertility outcomes in female cancer patients: a review

Monografia realizada no âmbito da unidade Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pela Professora Doutora Ana Cristina Ribeiro Rama e apresentada à Faculdade de Farmácia da Universidade de Coimbra

Setembro 2015

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Coimbra, 7 de Setembro de 2015.

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(Cristiana Lopes Martinho)

O Tutor da Monografia

(Prof.ª Doutora Ana Cristina Ribeiro Rama)

A aluna

Cristiana Lopes Martinho

(Cristiana Lopes Martinho)

Agradecimentos

Porque nunca caminhamos sozinhos, gostaria de fazer um breve agradecimento às pessoas que me permitiram construir o meu percurso académico.

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INDEX

Efeitos da quimioterapia na reserva ovárica e fertilidade em mulheres com cancro: uma revisão.

RESUMO

Com o melhoramento contínuo da eficácia dos agentes antineoplásicos e das taxas de sobrevivência, tornou-se importante estudar os efeitos secundários a longo prazo, incluindo a fertilidade. Os agentes de quimioterapia induzem falência ovárica devido a danos causados em todos os tipos de folículos, desde primários a pré-antrais e antrais. É sabido que o grau do dano causado depende de um conjunto de fatores de entre eles a idade, o protocolo de tratamento e dosagem, podendo as mulheres recuperar ou não da Falência Ovárica Prematura. Em mulheres com cancro, marcadores hormonais e ultrassonográficos têm sido investigados como possíveis marcadores de reserva ovárica, permitindo assim um aconselhamento médico individualizado e afetando significativamente as decisões de tratamento no que respeita à subsequente função reprodutiva.

Objetivo: O nosso objetivo é compilar toda a informação científica possível relativa aos efeitos da quimioterapia nos marcadores de reserva ovárica. Esta informação irá permitir que doentes e médicos identifiquem mais facilmente os riscos de infertilidade, suportando assim decisões informadas no que respeita à preservação da fertilidade.

Métodos: Uma revisão da literatura publicada desde 1990 foi feita recorrendo ao Medline, através da PubMed. Os artigos eram selecionados se avaliassem o impacto de tratamentos antineoplásicos em diferentes marcadores de reserva ovárica, tanto hormonais como ultrassonográficos. Os estudos foram caracterizados e foi feita uma compilação dos resultados, organizada por doença oncológica e protocolo de quimioterapia.

Resultados: As doentes não só apresentaram níveis significativamente baixos de AMH, Inibina, FSH/LH e Estradiol após a quimioterapia, como também de volume ovárico e contagem de folículos antrais. Estudos observacionais destas variações fornecem informação útil acerca do grau do dano folicular para cada doente e de acordo com diferentes protocolos de quimioterapia.

Conclusões: A quimioterapia está correlacionada com a indução de danos no ovário, e os marcadores de reserva ovárica aparecem diminuídos em mulheres sujeitas a terapia anti-neoplásica. A avaliação hormonal e ultrassonográfica podem assistir decisões médicas no que concerne tratamentos opcionais e métodos de preservação da fertilidade, oferecendo uma oportunidade considerável de melhoria dos tratamentos de doenças malignas na mulher.

Effects of chemotherapy on ovarian reserve and fertility outcomes in female cancer patients: a review.

ABSTRACT

Background: with the continuous improvement of the efficacy of chemotherapeutic agents and cancer survival rates, it became important to acknowledge the long term effects of chemotherapy, including issues like fertility. Chemotherapy agents induce ovarian failure due to the damage caused in all kinds of follicles, from primary to preantral and antral. It is well known that the degree of the damage is also dependent of age, treatment protocol and dosage and therefore women may or may not recover from Premature Ovarian Failure (POF), depending on these aspects altogether. In women with cancer, hormonal and ultrasonographic markers such as AMH, Inhibin A and B, FSH/LH, Estradiol and Ovarian volume and Antral Follicle Count, respectively, have been investigated as presumably valuable predictors of ovarian reserve, thus improving individualization of advice to women facing chemotherapy and significantly affecting treatment decisions related to subsequent reproductive function.

Purpose: Our objective is to compile all published information concerning the effects of chemotherapy in ovarian reserve markers. This information will allow patients and physicians to better identify infertility risks, thus supporting informed decisions regarding fertility preservation.

Methods: A review of the literature published since 1990 was completed by searching Medline, through PubMed. Articles were selected if they assessed the impact of antineoplastic treatments in different Ovarian Reserve (OR) markers, both hormonal and ultrasonographic. The studies were characterized and a compilation of the results was made, which were organized by cancer disease and chemotherapy protocol.

Results: Cancer patients have not only significantly lower AMH, Inhibin, FSH/LH and Estradiol levels after chemotherapy, but also lower Ovarian Volume and Antral Follicle Count. Observational studies of these variations provide useful information about the degree of follicle loss for each patient according to different chemotherapy regimen.

Conclusions: Chemotherapy treatment may induce ovarian damage and measures of ovarian reserve appear to be diminished in female cancer survivors. Hormonal and ultrasonography assessment may assist decision-making regarding treatment options and fertility preservation procedures and offers considerable opportunity for the improved care of women malignancies.

INTRODUCTION

Advances in anticancer therapy have led to improved survival rates, posing emphasis on preserving an optimal quality of life after cancer therapy[1], where fertility preservation and parenthood have become an essential part of the equation.

Infertility represents one of the main long-term consequences of chemotherapy due to the gonadotoxic effect of anti-neoplastic agents although they are diverse and not yet absolutely understood[2]. Ovarian damage can be permanent, leading to premature ovarian failure and/or infertility, particularly in the case of protocols including alkylating agents, but recovery of ovarian function may also occur[3].

The magnitude of the gonadotoxic effects depends on women's age and type and dose of the chemotherapy regimen, but also on the number of existing primordial follicles, the so called ovarian reserve[2]. Having in mind the large variation in follicle number among women, even of the same age, there is a need for a reliable marker of future (in)fertility that will improve individualization of advice to women facing chemotherapy treatments allowing shared and informed decisions regarding fertility preservation and future reproductive function[4].

Studies analysing ovarian function status following cancer therapy have mostly described the prevalence of ovarian failure following treatment[5], based on markers such as cycle length, presence of amenorrhea and, in less extent, pregnancy occurrence. Nevertheless, studies have shown that follicular depletion may occur despite maintenance of regular menstrual cycles[4-7] and, on the other hand, the presence of amenorrhea is not an absolute marker of future infertility, as many women recover their ovarian function up to 2 years after finishing chemotherapy. Thus, more accurate indicators are needed in order to properly inform women about their risk of infertility after treatment.

Ovarian reserve can indirectly be assessed by several markers, hormonal and ultrasonographic. There is now a substantial body of evidence indicating that serum measurement of Anti-Müllerian Hormone (AMH; normal range 1.5 – 4.0 ng/ml) is a clinically useful biomarker of the ovarian reserve. In women, AMH appears to be exclusively of ovarian origin since it is undetectable in the serum 3 to 5 days after bilateral ovariectomy[8], and progressively increase from almost undetectable levels at birth to adult levels. It appears to be stable up to 25-30 years and then decrease throughout the reproductive life, until being undetectable after spontaneous menopause[8]. AMH is expressed by granulosa cells of follicles on initiation of growth until the early antral stages, thus potentially more closely reflecting the follicular reserve than other markers[5].

Moreover, as AMH concentration does not change significantly during the menstrual cycle[8] and is weakly influenced by short term gonadotropin suppressing treatments[9], unlike other follicular markers such as FSH, LH, estradiol, Inhibin B and Antral Follicle Count, it is considered as the most specific and reproducible marker of the ovarian reserve[3].

In this review, FSH, LH, Inhibin A and B, Antral Follicle Count and Ovarian Volume are also addressed. FSH (normal range during follicular phase: 3.1-7.9 mIU/mL, ovulation peak: 2.3-18.5 mIU/mL, luteal phase: 1.4-5.5 mIU/mL) and LH (normal range: 5 – 25 IU/L) strongly predict poor ovarian reserve in older women when elevated, but less so in younger women, thus not being nowadays the more accurate predictor of chemotherapy induced ovarian failure. Inhibin A (normal range: <97.5 pg/mL) and B (normal range during ollicular phase: <139 pg/mL; luteal phase: <92 pg/mL) are secreted by developing antral follicles in females, and may be a marker of gonadal function in prepubertal children[10]. However, it is subject to vast cyclic variation, limiting its use as a marker of gonadotoxicity in children and adolescents[11]. While both the Ovarian Volume and the AFC (normal range: 30 by 18mm per ovary and 22 – 35 follicles, respectively), which can be measured by ultrasonography methods, reliably reflect the size of the remaining primordial follicle pool, i.e. the ovarian reserve[6], they are less readily available and require expertise to maximize accuracy[4].

Our objective is to compile all published information concerning the effects of chemotherapy in ovarian reserve markers. This information will allow patients and physicians to better identify infertility risks, thus supporting informed decisions regarding fertility preservation.

MATERIALS AND METHODS

A review of the literature published since 1990 was completed by searching Medline, through PubMed. The search equation combined MeSH and non-MeSH terms related with chemotherapy/antineoplastic treatments, in one hand, and ovarian function and corresponding markers, on the other (search equation – Annex 1). Investigational studies were selected and meta-analysis, case reports and case series were excluded. The results were limited to studies in humans published from 1990 till May 2015.

Articles analyzing and describing the effects of chemotherapy in hormonal and ultrasound ovarian reserve measures, such as AMH, FSH, LH, Inhibin or Estradiol levels, Antral Follicle Count or Ovarian Volume, were identified. Furthermore, articles had to describe patients' cancer type and the administered chemotherapy protocol and associate these variables with ovarian reserve markers. Articles were analysed in terms of setting,

study design, number of patients, age at diagnosis, follow-up and measurements points, ovarian markers assessed and cancer type and stage and a compilation of the results was made, organized by cancer disease and chemotherapy protocol.

RESULTS

From the search equation, 525 articles were obtained. From these, 32 were selected for further analysis. Nine of these were excluded because amenorrhea and/or pregnancy were the primary endpoints for defining chemotherapy related premature ovarian failure and infertility. Other eight articles were excluded due to the lack of information concerning chemotherapy protocol and/or cancer type. In the end, fifteen articles were considered for this review, all being observational studies. A flow diagram of the literature search is presented in Figure 1 and characteristics of the included studies are presented in Annex II.

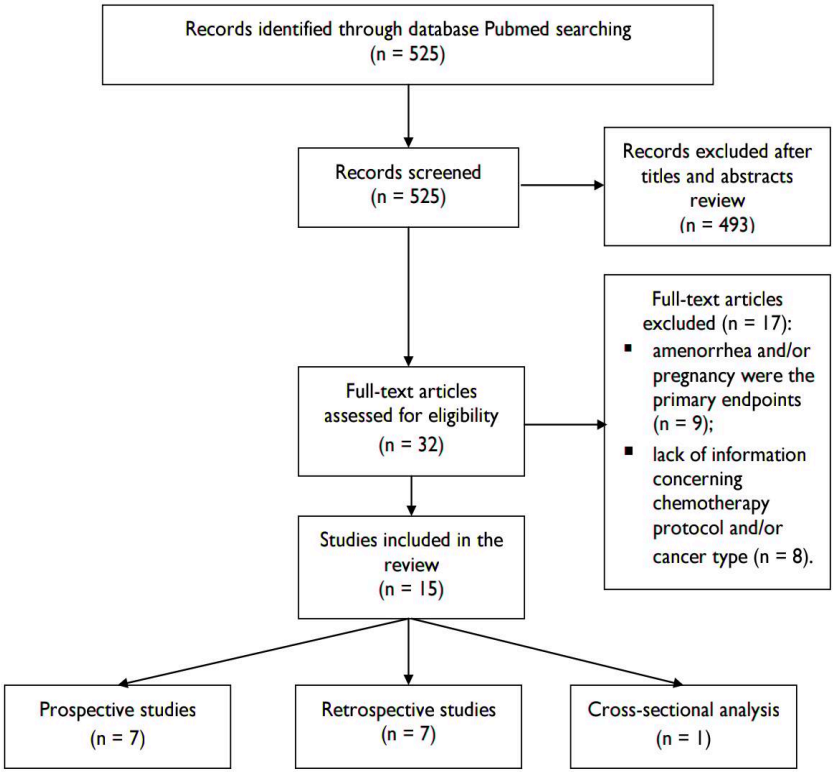

Figure 1. Flow diagram of the literature search.

From these fifteen, five studies included patients diagnosed with several types of cancer. Other five studies were conducted in Hodgkin's Lymphoma patients (one of them including non-Hodgkin's Lymphoma patients), two in breast cancer patients, one in Gestational Trophoblastic Neoplasia patients, one in Sarcoma patients and one in Acute Myeloid Leukemia patients.

Studies addressing several types of cancer

Significantly decreased or undetectable AMH levels at the end of chemotherapy were found in studies conducted in women with several types of cancer and chemotherapy protocols[6,12-15].

E. C. Larsen et al. [6] described the impact of **alkylating agents** in a cohort of 100 childhood cancer survivors (mean age at diagnosis: 5.4), diagnosed with different types of cancer (47 acute lymphoblastic leukemia, 2 acute myeloid leukemia, 1 chronic myeloid leukemia, 6 non-Hodgkin lymphoma, 7 Hodgkin's disease, 18 Wilms' tumor, 9 neuroblastoma, 2 Ewing's sarcoma, 5 soft-tissue sarcoma, 1 osteosarcoma, 2 teratoma), having 21 controls of similar age as comparators. Patients were divided into three groups: Group 1 consisted of 35 women treated with non-alkylating chemotherapy, 3 of whom also received cranial irradiation; Group 2 consisted of 55 women either treated with alkylating agents, with alkylating agents and radiotherapy above the diaphragm, with alkylating agents and radiotherapy below the diaphragm or with radiotherapy below the diaphragm and non-alkylating agents; and Group 3 consisted of 10 women treated with TBI and cyclophosphamide before BMT. Results showed a significant difference solely regarding Ovarian Volume and Antral Follicle Count. The control group had significantly larger ovaries and a significantly higher number of total Antral Follicles than both group 1 and group 2 survivors, and between these two, group 1 showed larger ovaries and higher AFC. Group 3 showed the worse results of the three groups, although not significant. When Group 2 subgroups were analysed, there was no significant difference in between them. In regard to hormones, there were not found significant differences concerning AMH levels. Only FSH showed significant differences, being higher in group 3 than in 1 and 2. Survivors with preserved menstrual cycles have ultrasonographic and endocrine changes, suggesting impairment of ovarian potential and an advanced ovarian age. This data shows that regular menstrual cycles can erroneously indicate a normal ovarian potential. Both ovarian volume and AFC were lower than in the controls, showing that the biological ovarian age in childhood cancer survivors could be approximately 10 yr ahead of their chronological age [Table 1].

R. Gracia et al.[12] included 71 survivors (mean age: 25.7) and 67 similarly aged controls. Twenty-four of the survivors were treated for lymphoma (15 Hodgkin, 9 non-Hodgkin), 23 for leukemia, 10 for sarcoma, 4 for Wilms' tumor, 3 for breast cancer, and 7 for other types of cancer and 67 similarly aged controls. Chemotherapies were categorized based on the **alkylating agents** included, such as carmustine, lumustine, busulfan, chlorambucil, cyclophosphamide, ifosfamide, melphalan, nitrogen mustard, procarbazine, and thiotepa. The

Alkylating Agent Dose scores (range 0-9) were determined by assigning a score ranging from 1 to 3 for each agent received, and summing the scores over each agent received. Exposure to pelvic radiation was defined as exposure to direct pelvic radiation or total body irradiation and happened in 83% of the cohort. Results showed that, for each unit increase in Alkylating Agent Dose, FSH increased by 0.91mIU/ml and AMH decreased by 0.55 ng/ml and the comparison between survivors and the control group showed a significant impairment, FSH being higher and AMH, AFC, Ovarian and Uterine Volumes lower in survivors. Moreover, a dose-dependent relationship between cancer therapies and measures of ovarian reserve was also noted and cancer survivors with greater exposure to alkylators, pelvic radiotherapy, or BMT with TBI had the most impaired ovarian reserve. Interestingly, even among cancer survivors with regular menstrual cycles or those with FSH levels considered to be in the normal range, AMH and AFC were significantly lower compared to unexposed females of similar age, supporting sub-clinical follicular depletion [Table 1].

Mörse et al.[13] demonstrated that chemotherapy cause immediate and profound ovarian dysfunction in prepubertal and pubertal females. He described the results in 34 females (mean age: 9.5; only 11 patients had reached menarche), 8 with Acute Lymphatic Leukemia, 3 with Acute Myeloblastic Leukemia, 4 with Hodgkin Lymphoma, 3 with Wilms' tumor, 7 with Ewingsarcoma or Osteosarcoma, 4 with Brain tumors and 5 with other diagnoses. Treatment regimen included **alkylating agents** for 27 out of 34 patients, who were grouped in low/median or high risk groups based on cumulative dose of alkylating agents and radiotherapy involving the ovaries. All patients had detectable AMH levels at diagnosis and they all showed a rapid decline in AMH after 3 months of treatment. Inhibin B was detectable at diagnosis only in 16/34 patients and was below the detection limit in 29/34 patients after 3 months, independently of the initial levels. FSH and LH increased after 3 months. Patients who required radiotherapy below the diaphragm and/or stem cell transplantation showed no recovery of ovarian failure during and up to one and half years after treatment. This study shows that AMH is a relevant marker of ovarian function in young females before and after menarche [Table 1].

In another study, *Krawczuk-Rybak et al.*[14] divided 33 survivors in two groups according to the type of malignancy and treatment and compared the results with 34 healthy controls. High risk group included Hodgkin Lymphoma, Soft Tissue Sarcoma patients and patients who needed BMT and the chemotherapy protocol was as follows: HL patients received **MVPP** and **B-DOPA**; BMT underwent megachemotherapy of **busulphan** and **cyclophosphamide** and Sarcoma patients were given **Ifosfamide (Actinomycin D, Vincristine), Dacarbazine** and **Cyclophosphamide**. Low/medium risk group included

patients diagnosed with Wilms' tumour, Neuroblastoma (stage II), Germ tumours, and Soft Tissue Sarcoma and chemotherapy also included **Ifosfamide**, **Actinomycin D, Vincristine, and Cisplatin**. Two examinations were performed, the first one 6-11 years after treatment termination and the second 4-6 years after the first. High risk group revealed significantly lower AMH values than in controls and low/medium risk group at the first examination, and fell progressively after 5 years. FSH values increased and unchanged LH values in the first examination rose in the second one in the HR group. Estradiol was also measured and showed no differences between control, high risk and low/medium risk groups and remained unchanged in the second examination. In which concerns the low/medium group, FSH values were normal in the first examination and did not change but AMH values decreased from 4.10 ng/ml to 2.68 ng/ml and were lower than in the control. These findings indicate a progressive deterioration of ovarian function and diminished ovarian reserve not only in the high risk group but also in the low/medium risk group [Table 1]. The group of survivors diagnosed with Hodgkin lymphoma was separated for further analysis [Table 2]. Normal values were found in the first analysis whereas in the second analysis a difference was observed compared to the control, AMH was lower (1.26 ng/ml) and FSH increased to 9.64 mIU/ml. Also, patients diagnosed with solid tumors in the high risk group were found to have lower AMH values than those in the low/medium group at the time of the first examination (0.50 ng/ml vs. 4.64 ng/ml), and a tendency to lower values in the second examination (0.65 ng/ml vs. 2.67 ng/ml) [Table 5]. LH values were elevated in the high risk group at the time of the second examination (27.75 mIU/ml vs. 5.08 mIU/ml) [Table 5].

Katherine E. Dillon, et al.[15] also aimed to study the impact of **alkylators**. 46 women (mean age: 26.1) with different types of cancer were included, 19 with Breast Cancer, who were given TCH, TC, Actinomycin-D or ECT, 4 with Leukemia and 13 Lymphoma, which were given BEACOPP, ABVD, ABVE, R-CHOP or ICE, 4 with Sarcoma, 1 with Brain Tumor, 1 with Wilms' Tumor, 2 with Germ Cell's Tumor and 2 with other types. Exposure to alkylating agents was significantly associated with post-therapy impairment of AMH, Estradiol, Inhibin and AFC - these patients showed higher post-treatment FSH and LH levels and lower AMH, Estradiol and AFCs than those who did not receive alkylators [Table 1]. This study interestingly showed that the rate of recovery for AMH was significantly impacted by pre-treatment AMH levels: participants who began with pre-treatment AMH levels < 2.0ng/mL had a rate of recovery of 2.6% per month, whereas for those starting with pre-treatment AMH levels ≥2.0ng/mL, recovery rate was 11.9% per month.

Table 1. Effects of chemotherapy in patients diagnosed with several types of cancer.

Author	Cancer type	CT Regimen	Markers assessed	Results
E. C. Larsen et al. [6]	Acute lymphoblastic leukemia Acute myeloid leukemia Chronic myeloid leukemia Non-Hodgkin lymphoma Hodgkin's lymphoma Wilms' tumor Neuroblastoma Ewing's sarcoma Soft-tissue sarcoma Osteosarcoma Teratoma	***Group 1:*** asparginase, methotrexate, steroids, 6-mercaptopurine, thioguanine, vincristine, dactinomycin, and doxorubicin. ***Group 2:*** cyclophosphamide, ifosfamide, nitrosoureas, busulphan, melphalan, mustagene, chlorambucil, dacarbazine, procarbazine, cisplatinum, vinblastine, cytarabine, bleomycin, adriablastine, daunorubicin, vepeside, and VM-26. ***Group 3:*** cyclophosphamide before BMT.	FSH LH* Estradiol* Inhibin A and B* Ovarian Volume and AFC	Group 1: larger Ovarian Volume and higher AFC than group 2. Group 3: only FSH showed significant difference, being higher in Group 3.
R. Gracia et al. [12]	Hodgkin non-Hodgkin Lymphoma Leukemia Sarcoma Wilms' tumor Breast cancer	**AAD w/o radiation or TBI	FSH Estradiol* Inhibin B* AMH Ovarian Volume and AFC*	FSH: increase in 0.91mIU/ml for each unit increase in AAD. AMH: decrease in 0.55 ng/ml for each unit increase in AAD.
Mörse et al. [13]	Acute lymphatic leukemia Acute myeloblastic leukemia Hodgkin lymphoma Wilms' tumor Ewingsarcoma Osteosarcoma Brain tumors	Alkylating agents in 27 out of 34 patients	AMH Inibin B FSH* LH* Estradiol	AMH: rapid decline after 3 months of treatment. Inhibin B: detectable at diagnosis 16 females; below the detection limit in 29 patients after 3 months of treatment. FSH and LH increased after 3 months of treatment.
Krawczuk-Rybak et al. [14]	Hodgkin lymphoma Soft tissue sarcoma Wilms' tumour Neuroblastoma (stage II) Germ tumours	***High risk:*** MVPP and B-DOPA; busulphan and cyclophosphamide; Ifosfamide (Actinomycin D, Vincristine), Dacarbazine, Cyclophosphamide. ***Low risk:*** Ifosfamide, Actinomycin D, Vincristine, Cisplatin.	AMH FSH LH* Estradiol*	***High risk:*** AMH: 1st examination: 2.0 ng/ml; after 5 yrs: 0.96ng/ml. FSH: 1st examination: 19.53 mIU/ml. 2nd examination: 29.41 mIU/ml; ***Low risk:*** AMH: 1st examination: 4.10 ng/ml. 2nd examination: 2.68 ng/ml. FSH: showed normal levels in the 1st examination and did not change.
Katherine E. Dillon, et al. [15]	Breast cancer, Leukemia, Lymphoma, Sarcoma, Brain tumor, Wilm's Tumor, Germ Cell tumor.	TCH TC ACT ECT BEACOPP ABVD ABVE R-CHOP ICE	FSH* LH* Estradiol Inhibin B AMH Ovarian volume and AFC	AMH levels 76% lower, E2 13% lower, Inhibin 54% lower, and AFC 59% lower at the end of chemotherapy compared to unexposed participants. ***Rate of recovery*** : impacted by pre-treatment AMH levels. - pretreatment AMH levels < 2.0ng/mL: 2.6% rate of recovery per month. - pretreatment AMH levels ≥2.0ng/mL, 11.9% rate of recovery per month.

* Markers not mentioned in the Results did not reach statistical difference.

** AAD – Alkylating Agents Dose scores (determined by assigning a score ranging from 1 to 3 for each agent received, and summing the scores over each agent received)
MVPP – nitrosurea, vinblastine, procarbazine and prednisolone
B-DOPA – bleomycin, dacarbazine, vincristine, adriamycin and prednisone/prednisolone
TCH – docetaxel, carboplatin, trastuzumab
TC – docetaxel, cyclophosphamide
ACT – doxorubicin, cyclophosphamide, taxane
ECT – epirubicin, cyclophosphamide, taxane
BEACOPP – bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone
ABVD – doxorubicin (adriamycin), vinblastine, dacarbazine, bleomycin
ABVE – doxorubicin, bleomycin, vincristine, etoposide
CHOP – cyclophosphamide, adriamycin, vincristine, prednisolone
ICE – ifosfamide, carboplatin, etoposide

Studies on Hodgkin's Lymphoma

Five of the 15 articles analysed included women diagnosed with Hodgkin's Disease (HD)[16-20], one of those also including non-Hodgkin patients[22]. For the purpose of this study, only the findings concerning women patients are discussed, although all the five articles included both genders [Table 2].

Behringer et al. [16] studied 562 women (mean age: 32) after therapy within the German Hodgkin Study Group HD13 to HD15 trials for early and advanced-stage HD. Chemotherapy consisted of HD13, 2 cycles of **ABVD** with or without bleomycin for early stage HD (IA or IB), HD14, which included in arm A: 4 cycles of ABVD and in arm B: 2 cycles of escalated **BEACOPP** + 2 cycles of ABVD and HD15, 6 or 8 cycles of escalated BEACOPP or 8 cycles of BEACOPP-14, for stages > II or early stage with unfavorable risk factors. In the HD13 group, differences in favor of patients treated with fewer cycles of less intensive chemotherapy were significant for AMH and FSH in both age groups and mean AMH levels greater than 2ng/mL were only observed in women younger than 30 years after ABVD therapy. In HD14 group, differences between arms were high and significant for FSH in older women and for AMH in both age groups. In respect to HD15 trial, no differences were found between the arms: mean AMH levels were 0ng/mL in both age groups, and the highest FSH levels were measured [Table 2]. As expected, chemotherapy-induced gonadal toxicity was highest after six to eight cycles of escalated BEACOPP. This prospective study allows the conclusion that hormone levels correlate with the intensity of chemotherapy, and that age is also a relevant factor.

In a different study, *K.Behringer et al.* [17] particularly described the HD14 Study Group (protocol described above). In a cohort of 117 women (arm A: 62; arm B: 55) with a mean age of 32 years, results show a difference in AMH and FSH between the arms. These differences in favor of ABVD were high and significant for FSH in older women and for AMH in both groups [Table 2]. Thus, hormonal levels of AMH and FSH demonstrate a distinct

difference between both treatment groups, in favor of arm A (ABVD). It is important to note that there were more pregnancies in the arm B, hence AMH levels were not conclusive on female fertility in this study. However, as decreased AMH levels indicate reduced follicule pool, a higher rate of future POF cannot be excluded and a longer follow-up period is needed.

Vassilios Papadakis et al. [18] evaluated the effect of chemotherapy, radiotherapy or both in 29 females (mean age: 14.1) with Hodgkin's Disease, aiming to compare **MDP** with COPP and ABVD protocols. Women were divided in groups based on the given treatment: group A, 8 women only received RT not including the pelvis; group B, 15 women received CT but not pelvic RT and group C, 6 women received CT plus pelvic RT. Chemotherapy was administered according to the MDP protocol which consisted of repeated cycles of **doxorubicin** in the 1st phase, **procarbazine** in combination with **prednisone** and **vincristine** during the 2nd phase, and **cyclophosphamide** in the 3rd phase and the ovarian function was assessed with measurements of FSH and LH serum concentrations. These markers were within the normal range at the time of the last evaluation in 8/8 group A patients, 12/15 group B patients and 4/6 group C patients indicating a 17% prevalence of ovarian dysfunction. This study allowed the conclusion that females following the MDP protocol had similar FSH/LH levels to what has been noted in girls treated with COPP or ABVD [Table 2]. Interestingly, six females delivered eight normal children, showing that, although abnormal function early after the end of treatment was observed, ovarian function remained or returned to normal in most young women.

In another study, *E. D. Kreuser et al.* [19] evaluated the effects of the combination of **COPP/ABVD** in 22 women with Hodkin's Disease (mean age: 35) by proceeding to FSH and LH measurements. Results showed that 17/22 women exhibited increased FSH and LH levels 2-8 yrs after chemotherapy, indicating premature ovarian failure and 5/22 women retained normal ovarian function, all being under 30 at the time of treatment. The most important finding was the high incidence of gonadal impairment with this combination. There is evidence of minimal sterility risk in females receiving the ABVD regimen alone, and this study showed that combined COPP and ABVD regimens can produce significant and irreversible ovarian failure [Table 2].

Z. Blumenfeld et al. [20] included both Hodgkin's and non-Hodgkin's disease patients and described the impact of different chemotherapy protocols in 22 women (mean age: 25.8), aiming to study not only the variations in Inhibin A, but also the effectiveness of GnRH-agonists as inhibitors of chemotherapy-induced ovarian follicular depletion. However, for the purpose of this article, only the control group (22 patients that did not receive GnRH-a)

results are presented and the changes in hormonal levels exposed. Chemotherapy regimens for this group included **COPP/ABVD**, **CHOPP**, **(cyclophosphamide)-MOPP**, **ABV** only and chemotherapy details for the other 2 women is not known. Levels of Inhibin A were low, compatible with menopausal levels, in women who had amenorrhea and increased thereafter in parallel with the return of ovarian cyclic activity, whereas women who resumed cyclic ovarian function had inhibin A within normal levels [Table 2]. Also, inhibin A levels in those who spontaneously conceived were higher than in those who did not.

Table 2. Effects of chemotherapy in patients diagnosed with several types of cancer.

Cancer type	CT Regimen	Markers assessed	Results
Hodgkin Lymphoma [14, 16-20]	MVPP and B-DOPA [14]	AMH FSH LH E2	***first examination*** (6-11 years after treatment): AMH 2.96 ng/ml ± 2.05, FSH 6.88 mIU/ml ± 3.41, LH 3.83 mIU/ml ± 2.12, E2 47.24 pg/ml ± 31.60. ***second examination*** (4-6 years after the first examination), AMH was lower (1.26 ng/ml ± 0.84), FSH increased to 9.64 mIU/ml ± 3.25.
	German Hodgkin Study Groups: [16] **HD13:** 2 x ABVD with or without bleomycin. **HD14**: armA: 4 x ABVD; armB: 2 x escalated BEACOPP + 2 x ABVD. **HD15:** 6-8 x escalated BEACOPP or 8 x BEACOPP-14.	FSH LH Estradiol* AMH Inhibin B*	**HD13:** Differences in favor of regimens with fewer cycles of less intensive chemotherapy were significant for AMH and FSH. AMH > 2ng/mL was only observed in women younger than 30. **HD14:** mean AMH in women < 30 years: arm A, 2.3 ng/ml; arm B: 0.9ng/ml; mean AMH in women > 30 years: arm A, 0.7ng/ml; arm B, 0.0 ng/ml; mean FSH in women 30 - 45 years: arm A, 4.4IU/L; arm B, 11.8 IU/L. **HD15:** mean AMH levels were 0 ng/ml in both age groups; the highest FSH levels were measured.
	German Hodgkin Study Group HD14: [17] **Arm A**: 4 cycles of ABVD **Arm B**: 2 cycles BEACOPP + 2 cycles ABVD	FSH LH* Estradiol* AMH	**AMH:** **Arm A**: 2.2 ng/ml (18-29 yrs); 0.8 ng/ml (30-45 yrs); **Arm B**: 0.9 ng/ml(18-29 yrs); 0.03 ng/ml (30-45 yrs). **FSH:** **Arm A**: 4.4 IU/l and B: 11.9 IU/l, in older women.
	Doxorubicin, Procarbazine, Prednisone, Vincristine and Cyclophosphamide. [18]	LH and FSH	20% of the females had increased FSH/LH levels.
	COPP/ABVD: [19] Cyclophosphamide, vincristine, procarbazine, prednisone, doxorubicin, bleomycin, vinblastine, dacarbazine.	FSH LH Estradiol*	17/22 women showed increased FSH and LH; 5/22 women retained normal ovarian function, all being ≤ 30 at the time of treatment.

	COPP (or C-COPP)/ABV (D) [20]	FSH LH Estradiol* Inhibin A	Mean FSH 3-6 months after completion of treatment: 37 IU/L. Mean LH 19.4 f 10.3 IU/L. Inhibin A levels were low in women who had amenorrhea. Inhibin A levels were normal in women who resumed cyclic ovarian function.
Non Hodgkin Lymphoma[20]	COPP (or C-COPP)/ABV (D)	FSH LH Estradiol* Inhibin A	Mean FSH 3-6 months after completion of treatment: 37 IU/L. Mean LH 19.4 f 10.3 IU/L. Inhibin A levels were low in women who had amenorrhea. Inhibin A levels were normal in women who resumed cyclic ovarian function.

* Markers not mentioned in the Results did not reach statistical difference.

Studies on Breast cancer

Two studies approached premenopausal women diagnosed with early stage Breast Cancer [5, 7].

R. A. Anderson et al. [5] analysed 50 premenopausal women (mean age: 41), of which 20 were enrolled in a trail of **accelerated chemotherapy** (TACT), 22 received chemotherapy without a trial and 8 did not receive chemotherapy and were given hormonal suppression with Gosereline and/or Tamoxifen. Accelerated chemotherapy consists of an optimization of the schedule of the treatments in order to improve the outcome, sometimes involving dosage increase, and in the TACT group chemotherapy protocols were **FEC-T**, **E-CMF** and **TANGO** and in the non-TACT group chemotherapy consisted of AC (doxorubicin + cyclophosphamide), CMF, A-CMF or E-CMF. Chemotherapy group showed a marked increase in FSH and LH within 3 months, which was sustained at 6 months, and no significant change in Estradiol. AMH and Inhibin B showed consistent and rapid falls – AMH showed concentrations close to the limit of detection in many women and Inhibin B fell to 50% of pretreatment concentration by 3 months and became undetectable in 20/42 women at 6 months. Within chemotherapy group, results showed highest FSH and lowest Estradiol and AMH concentrations after treatment in the FEC-T (TACT) group, this being the only group in which a fall in Estradiol concentration occurred. E-CMF (TACT) group showed similarly low AMH concentrations (i.e. at the limit of detection in all women) but was similar to the other two groups in FSH and E2 concentrations. Both Ovarian Volume and AFC also fell, although these changes were modest [Table 3]. A fall of 20% in AMH concentrations would be expected from the effects of increasing age alone, compared with the 70% observed. These change in AMH concentrations were shown to indicate gonadal toxicity during chemotherapy for breast cancer more clearly than those in Estradiol and Inhibin B, supporting a role for AMH as a marker of ovarian damage during such therapies.

R. A. Anderson et al. [7] also studied 33 women, treated with **neo-adjuvant adriamycin** and **cyclophosphamide** or post-operative chemotherapy which consisted either of CMF, Anthracycline-CMF or Anthracyclines and taxanes. All ovarian hormones were lower during the 2-5 years after chemotherapy, and FSH was higher in 26/33 women. Inhibin B and AMH were very low throughout the study and were undetectable in most women. At 5yr, inhibin B was detectable in only 7 women and AMH in 6, with both hormones detectable in only 4 women [Table 3]. Furthermore, pretreatment AMH was significantly higher in women who had ongoing menses at 2-5 years after treatment than in women who were amenorrheic and all women with pretreatment AMH below 1.9 ng/ml became amenorrheic, also showing that serum AMH determined at the time of diagnosis is strongly predictive of long-term ovarian function after chemotherapy.

Table 3. Effects of chemotherapy in Breast cancer patients.

Cancer type	CT Regimen	Markers assessed	Results
Breast Cancer [5, 7]	FEC-T (TACT) E-CMF (TACT) E-CMF A-CMF [5]	FSH LH E2 Inhibin B AMH	***All CT regimens*** FSH and LH: marked increase within 3 months; sustained at 6 months. E2: no significant change, except for FEC-T group. Inhibin B: consistent and rapid fall to 50% of pretreatment concentration by 3 months; undetectable in 20/42 women at 6 months. AMH: Consistent and rapid fall; concentrations close to the limit of detection in many women. ***FEC-T (TACT) group*** Highest FSH and lowest E2 after treatment, and was the only group which showed a fall in E2 concentration. Lowest AMH concentrations after treatment.
	Neo-adjuvant adriamycin and cyclophosphamide; Post-operative chemotherapy was either CMF, Anthracycline-CMF or Anthracyclines and taxanes. [7]	Estradiol* Inhibin B FSH* AMH AFC* Ovarian Volume*	AMH: Very low throughout the study and undetectable in most women; detectable after 5 years in 6 women. Inhibin B: Very low throughout the study and undetectable in most women; detectable after 5 years in 7 women; AMH and Inhibin B were both detectable in only 4 women.

* Markers not mentioned in the Results did not reach statistical difference.

Gestational Trophoblastic Neoplasia

Akira Iwase et al. [21] assessed the chemotherapy impact in AMH marker in 22 women (mean age: 35.1) with Gestational Trophoblastic Neoplasia (GNT). To do so, women were divided into two groups: the low risk group received **Methotrexate** (MTX) and/or **Actinomycin D** (ACD) or **Etoposide** (ETP) if resistance to MTX and ACD; the high risk group received **Etoposide** (ETP). AMH was higher (1.30 μg/ml) in the low risk group, in comparison with the high risk group, where AMH decreased to 0.71 μg/ml. Low risk patients

resistant to MTX and ACD and, therefore, treated with ETP, also had decreased values of AMH in comparison to the other patients in this group. This data shows that chemotherapy administered to treat GTN does indeed affect the ovarian reserve, especially in patients who receive a medication regimen that includes Etoposide [Table 4].

Table 4. Effects of chemotherapy in Gestational Tromphoblastic Neoplasia cancer patients.

Cancer type	CT Regimen	Markers assessed	Results
Gestational Trophoblastic Neoplasia [21]	***Low risk:*** MTX and/or ACD; Etoposide if resistence to MTX and ACD. ***High risk:*** ETP	AMH	***Low risk:*** 1.30 ng/ml. ***Hish risk:*** 0.71 ng/ml. ***Low risk treated with ETP***: 0.71 ng/ml.

MTX, Methrotexate; ACD, Actinomycin-D; ETP, Etoposide;

Sarcoma

Denise Williams et al. [22] described the effects protocols containing **ifosfamide** (median dose 59.4 g/m^2) in 13 girls (mean age: 12.1) diagnosed with Sarcoma. Median AMH levels were lower in the study group, closer to that reported for older women aged 30-35 years. There was no correlation between the dose of ifosfamide and hormone levels. Nevertheless, these results are consistent with depletion of follicle reserve, which may result in a premature menopause [Table 5].

Table 5. Effects of Ifosfamide in Sarcoma cancer patients.

Cancer type	CT Regimen	Markers assessed	Results
Sarcoma [14, 22]	Ifosfamide [14]	AMH FSH* LH* Estradiol*	AMH, 1st examination: 4.64 ng/ml; 2nd examination: 2.65 ng/ml. LH, 2nd examination: 5.08 mIU/ml.
	Ifosfamide, (Actinomycin D, Vincristine), Dacarbazine, Cyclophosphamide [14]	AMH FSH* LH Estradiol*	AMH, 1st examination: 0.50 ng/ml; 2nd examination: 0.65 ng/ml. LH, 2nd examination: 27.75 mIU/ml.
	Ifosfamide [22]	AMH	Lower in the study group; no correlation between the dose of ifosfamide and hormone levels.

* Markers not mentioned in the Results did not reach statistical difference.

Acute Myeloid Leukemia

Molgaard-Hansen et al. [23] study included 56 females with Acute Myeloid Leukemia (mean age: 16; only 40 had reach menarche) and 51 siblings as controls, that were treated with **Cytarabin**, **Anthracycline**, **6-Thioguanine**, **Etoposide** plus intrathecal

Methothrexate given with each course. Survivors had normal pubertal development and fertility, although AMH levels decreased in 4/40 of postpubertal females. Other hormones were within the normal ranges for all females and none of the patients were treated with alkylators [Table 6].

Table 6. Effects of chemotherapy in Acute Myeloid Leukemia patients.

Cancer type	CT Regimen	Markers assessed	Results
Acute Myeloid Leukemia [23]	Cytarabin, Anthracycline, 6-Thioguanine, Etoposide + intrathecal Methotrexate	FSH* LH* Estradiol* AMH inhibin B*	AMH decreased in 5/40 portpubertal females. Other hormones were within the normal ranges for all females.

* Markers not mentioned in the Results did not reach statistical difference.

DISCUSSION

Recent diagnostic and therapeutic advances in oncology have led to greater survival rates in young female cancer patients, but these treatments may deplete the ovarian follicular pool increasing the risk of ovarian failure and infertility[12]. Accurate predictive markers could have clinical applicability in choosing between chemotherapeutic agents and also in deciding whether or not to use fertility preservation methods[21].

Analyses of ovarian function following cancer therapy have mostly described the prevalence of ovarian failure following treatment[5] focusing on markers such as cycle length, amenorrhea and/or pregnancy occurrence. Several studies also show a relation between AMH and amenorrhea, stating that the first is decreased in the majority cases of menses depletion. However, follicular depletion may occur despite maintenance of regular menstrual cycles[4-7], thus the need of more accurate indicators to properly inform women about their fertility after chemotherapy treatment.

In cancer survivors with regular menstrual cycles, markers of ovarian function may sometimes be lower, supporting a sub-clinical follicular depletion[6,12]. In these cases, AMH pays an important role, as *R. Garcia et al.*[12] shows in his study, where women with regular menstrual cycles had FSH levels within the normal range but AMH and AFC were significantly lower compared to unexposed females of similar age, supporting the importance of these measurements to estimate the ovarian follicle pool. Similar results are reported by *R. A. Anderson et al.*[5] where a 70% fall in AMH was observed, compared with the expected

20% fall caused by increasing age alone, supporting that the changes in AMH concentrations indicate gonadal toxicity more clearly than those in Estradiol and Inhibin B. This statement supports once again a role for AMH as a marker of ovarian damage during chemotherapy[5].

Additionally, *Mörse et al.* [13] demonstrated the difference in relevancy between AMH and Inhibin B in a cohort of prepubertal and pubertal females. All girls had detectable AMH levels at diagnosis and they all showed a rapid decline after three months of treatment, whereas Inhibin B was detectable at diagnosis in only 16/34 patients and was below the limit of detection in 29/34 patients after three months of treatment. These differences demonstrate that AMH is also relevant in young females before and after menarche. Yet in another study, *Z. Blumenfeld et al.*[20] shows that Inhibin A serve as an additional means for evaluation of ovarian function. Inhibin A concentrations decreased during chemotherapy treatment but increased to normal levels in patients who resumed ovarian ciclycity, compared with low levels in menopausal women and women who had developed POF. Although a larger group would be needed, this marker may also serve as a prognostic factor for predicting the resumption of ovarian function[20].

AMH is expressed by granulosa cells of follicles on initiation of growth until the early antral stages[5] and its concentration does not change significantly during the menstrual cycle[8]. Consequently, AMH has been considered particularly sensitive and convenient marker of follicular depletion in young women, during and after chemotherapy. Moreover, this hormone allows the distinction in ovarian toxicity between chemotherapy protocols and can be used as a predictor of ovarian function recovery, by measuring pre-treatment serum levels[4, 7, 15].

For the purpose of this review, we have considered 15 articles that analysed the impact of chemotherapeutic agents on several markers. As seen, some of these markers, namely AMH and AFC, may be better predictors of that impact. These results will contribute to a better, as more reliable, estimation of infertility risks in cancer patients, adding to the evidence regarding the effects of antineoplastic agents on the occurrence of amenorrhea.

All studies revealed significant impairment in the ovarian reserve markers FSH, LH, Inhibin B, AMH and/or AFC in cancer survivors.

Alkylating agents (AA) are particularly known to be gonadotoxic, due to its direct and dose-dependent destruction of oocytes and follicular depletion[2]. *R. Garcia et al.*[12] results corroborate this statement, as a significant increase in FSH and decrease in AMH occurs for each unit increase of AA. *E. Dillon et al.*[15] also found a significant correlation between the exposure to AA and post-therapy impairment of AMH, E_2, Inhibin and AFC, where exposed

patients had their levels 76%, 13%, 54% and 59% lower, respectively, compared with unexposed patients.

However, in another study describing the impact of these agents, *E. C. Larsen et al.*[6] did not find a significant difference concerning AMH levels, and a significant difference was found solely in ovarian volume and AFC. Because our endpoint was not only whether or not an AMH fall had occurred but also the degree of ovarian follicular depletion in cancer survivors, these results are not to be underrated. Alkylating chemotherapy and direct ovarian irradiation were the most important predictors of the total antral follicle count, and these results also suggest that treatment modalities should be taken into account if female survivors seek fertility counseling. Surprisingly, patients who had not received alkylating chemotherapy or irradiation below the diaphragm, had significantly smaller ovaries and fewer antral follicles than the control group, suggesting that **non-alkylating** chemotherapy has also some gonadotoxic effect[6].

The fact that the rate of recovery of AMH is impacted by pretreatment levels should also be considered during pre-treatment fertility preservation counseling. *E. Dillon et al.*[15] showed that participants who began the treatment with AMH levels below 2 ng/ml recovered only 2.6% per months, while patients who had pre-treatment AMH above 2 ng/ml had a recovery rate of 11.9% per month and the same conclusion was taken by *R. A. Richards et al.*[4,7], where pre-treatment AMH was significantly higher in women who had ongoing menses at 2-5 years after treatment compared with women who were amenorrheic. Thus, it is safe to say that pretreatment measurement of AMH shall be a useful predictor of long term post-chemotherapy loss of ovarian function, adding significantly to the only previously established individualizing predictor, i.e. age[4].

Post-treatment FSH and LH levels were studied by *Vassilios Papadakis et al.*[18] and *E. D. Kreuser et al.*[19]. If one shows that females following MDP protocol had similarly changes levels of FSH/LH levels to those following COPP or ABVD[18], the other concludes that the combination of COPP and ABVD can produce a significant and irreversible ovarian failure when compared with the risk of taking ABVD alone[19]. These studies are therefore considered important when acknowledging the use of COPP and ABVD altogether.

Additionally, *Molgaard-Hansen et al.*[23], in a study of Acute Myeloid Leukemia survivors treated with non-alkylating chemotherapy came to the conclusion that women had reproductive hormones within the normal range, hence a normal pubertal development and fertility was observed, although AMH levels were decreased in 5/40 of post pubertal females. Longer follow-up is necessary to evaluate a possible risk of premature ovarian failure, but

this holds promising fertility outcomes for acute myeloid leukemia patients treated with Cytarabin, Anthracycline, 6-Thioguanine, Etoposide and Methothrexate.

The importance of ovarian reserve markers in considering different chemotherapy protocol options can also be seen with *Krawczuk-Rybak et al.*[14] results. AMH values appear to be lower and LH higher in patients treated with a combination of Ifosfamide, (Actinomycin D, Vincristine), Dacarbazine, and Cyclophosphamide, while patients treated exclusively with Ifosfamide do not show such decreased AMH and increased LH values.

Studies have interestingly reported few cases of cancer survivors that achieved pregnancy and delivered healthy children as well[17, 18], demonstrating that in young women the results of hormone testing performed early after treatment may not be predictive of their eventual reproductive potential[18]. This being said, further studies and longer follow-up times are needed to explain such discrepancies.

The main difficulty found during this review was the heterogeneity of the analysed articles, once they approached a wide number of different cancer types, chemotherapy protocols and hormonal and ultrasonographic measures. Furthermore, a few included a co-treatment group of women receiving GnRH agonists, that were tested as a fertility preservation strategy. Another critic that can be made is that, in many studies, the length of follow-up was not sufficient to reliably address all possible consequences of treatments in fertility.

Several limitations should be mentioned. Literature search was only conducted in Medline, which limited the number of articles analysed. Also, the authors of the excluded articles could have been contacted in order to acknowledge the information that was lacking. Moreover, a variety of diagnoses and treatments are included in this review, some with a limited sample size, which makes it difficult to address the effect of specific chemotherapeutic regimens on ovarian reserve.

The number of studies that address hormonal and ultrasonographic markers as ovarian reserve predictors of the impact of chemotherapy are still very limited. Absence or presence of amenorrhea is still the most common endpoint described. Thus, focusing on reliable hormonal and ultrasonographic evaluation, such as AMH and Antral Follicle Count, when studying women's (in)fertility, should be, in our view, a priority for the years to come.

Female cancer survivors should be informed of their risk of premature cessation of fertility, even in case of regular menstrual cycles, which can erroneously indicate a normal ovarian potential.

CONCLUSION

Chemotherapy treatment may induce ovarian damage and measures of ovarian reserve appear to be diminished in female cancer survivors. From this review we can also conclude that some hormonal markers are better that others in predicting (in)fertility outcomes and that AMH may be a promising marker along with ultrasonography assessment and that together they may assist decision-making regarding treatment options and fertility preservation procedures and offering considerable opportunity for the improved care of future consequences of women malignancies when they happen in a fertile age.

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ANNEX I - Search Equation

(((((((("Antineoplastic Agents"[Mesh] OR "Antineoplastic Combined Chemotherapy Protocols"[Mesh])))) AND ((("Primary Ovarian Insufficiency"[Mesh] OR "ovarian function" OR "ovary function" OR "ovarian reserve" OR "ovarian markers" OR "ovary failure" OR "ovarian failure" OR Ovary/drug effects[MeSH] OR Ovarian diseases/chemically induced[MeSH] OR "Ovary/injuries"[Mesh] OR "Ovary/physiology"[Mesh] OR "mullerian" OR "antral follicle" OR "inhibin" OR "ovarian volume" OR "FSH" OR Ovarian Function Tests[Mesh] OR "Follicle Stimulating Hormone"[Mesh] OR "Anti-Mullerian Hormone"[Mesh]))))) AND ("1990/01/01"[PDat] : "2015/12/31"[PDat])))) NOT review[Publication Type]) AND ((Clinical Trial[ptyp] OR Clinical Trial, Phase III[ptyp] OR Comparative Study[ptyp] OR Controlled Clinical Trial[ptyp] OR Evaluation Studies[ptyp] OR Randomized Controlled Trial[ptyp] OR Multicenter Study[ptyp] OR Research Support, N I H, Extramural[ptyp] OR Research Support, Non U S Gov't[ptyp] OR Research Support, U S Gov't, Non P H S[ptyp] OR Research Support, U S Gov't, P H S[ptyp] OR Research Support, N I H, Intramural[ptyp] OR Research Support, U.S. Government[ptyp] OR Observational Study[ptyp]) AND Humans[Mesh])

ANNEX II - Characteristics of the articles included in this review.

	2006, R. A. Anderson et al. [5]	***2003, E. C. Larsen, et al.*** [6]	***2011, A. Richard et al.*** [7]	***2012, R. Gracia et al.*** [12]	***2013, Mörse, et al.*** [13]	***2013, Krawczuk-Rybak et al.*** [14]
Setting	United Kingdom and The Netherlands	Denmark	United Kingdom	United States of America	Sweden	Poland
Study Design	Prospective	Controlled Prospective	Prospective	Cross-sectional controlled analysis of data	Prospective	Controlled Prospective
Number of participants	50	100	33	71	34	33
Mean age (range) in years	41 (28-52)	5.4 (0.1-15.3)		26.4 (15-39)	9.5 (4.5-16.5)	14
Follow-up and Measurement points	3, 6, 9 and 12 months after begining of chemotherapy		5 years; every 12 months		At diagnosis and every 3-4 months during and after treatment	1st examination: 6-11 years after treatment termination; 2nd examination: 4-6 years after the first one.
OR markers assessed	FSH, LH, Estradiol, Inhibin B, AMH; Ovarian volume, AFC.	FSH, LH, Estradiol, Inhibin A and B; Ovarian Volume and AFC	Estradiol, Inhibin B, FSH, AMH and AFC and Ovarian Volume	FSH, Estradiol, Inhibin B, AMH and Ovarian Volume and AFC	AMH, inibin B, FSH, LH, Estradiol	AMH, FSH, LH and Estradiol
Cancer type and stage	Breast cancer	Acute lymphoblastic leukemia, Acute myeloid leukemia, Chronic myeloid leukemia, Non-Hodgkin lymphoma, Hodgkin's lymphoma, Wilms' tumor, Neuroblastoma, Ewing's sarcoma, Soft-tissue sarcoma, Osteosarcoma, Teratoma	Breast cancer	Hodgkin non-Hodgkin Lymphoma, Leukemia, Sarcoma, Wilms' tumor, Breast cancer, other.	Acute lymphatic leukemia, Acute myeloblastic leukemia, Hodgkin lymphoma, Wilms' tumor, Ewingsarcoma or Osteosarcoma, Brain tumors, and other.	Hodgkin lymphoma, Soft tissue sarcoma, Wilms' tumour, Neuroblastoma (stage II), Germ tumours.

2013, Katherine E. Dillon et al. [15]	***2012, Behringer et al.*** [16]	***2012, Behringer et al.*** [17]	***1999, Vassilios Papadakis et al.*** [18]	***1992, E. D. Kreuser et al.*** [19]	***1998, Z. Blumenfeld et al.*** [20]	***2013, Akira Iwase et al.*** [21]	***2008, Denise Williams et al.*** [22]	***2013, Molgaard-Hansen et al.*** [23]
United States of America	Germany	Germany	United States of America	Germany	Israel	Japan	United Kingdom	Denmark, Finland, Iceland, Norway and Sweden
Prospective	Retrospective	Retrospective	Retrospective	Prospective	Retrospective	Retrospective	Retrospective	Controlled Retrospective
46	562	117	29	22	22	22	13	56
26.1 (15.0 – 35.9)	32 (18 - 39)	32 (18-40)	14.1 (6.1 ± 20.0)	35	25.8 (16 - 40)	35.1 (20 - 42)	12.1 (3.6–15.6)	16 (5 - 36)
Median of 12 months, every 3 months	46 months	Median of 42 months		Median of 5.3 years	Monthly after the start of chemotherapy until spontaneous ovulation or menses, and 3 to 6 months until 60	Monthly, every 1 to 42 months after chemotherapy	Median 10.1 years	Median of 11 years
FSH, LH, Estradiol, Inhibin B, AMH and ovarian volume and AFC	FSH, LH, estradiol, AMH, inibin B	FSH, LH, estradiol, AMH	FSH and LH	FSH, LH and Estradiol	FSH, LH, Estradiol and Inhibin A	AMH	LH, FSH, Inhibin B, AMH, Estradiol	FSH, LH, estradiol, AMH, inhibin B
Breast cancer, Leukemia, Lymphoma, Sarcoma, Brain tumor, Wilm's Tumor, Germ Cell tumor, Other.	Hodgkin Disease	Hodgkin Disease	Hodgkin's lymphoma	Hodgkin's lymphoma	Hodgkin and Non-Hodgkin's lymphoma	Gestational Trophoblastic Neoplasia	Sarcoma	Acute Myeloid Leukemia