Outcomes of ovarian stimulation after treatment with chemotherapy

Jessica L. Chan¹ · Lauren N. C. Johnson¹ · Brenda L. Efymow¹ · Mary D. Sammel² · Clarisa R. Gracia¹

Received: 16 July 2015 / Accepted: 7 September 2015 / Published online: 23 September 2015
© Springer Science+Business Media New York 2015

Abstract

Purpose Chemotherapeutic agents have a known gonadotoxic effect; however, it is difficult to predict the impact they may have on ovarian stimulation. The objective of this study was to evaluate response to ovarian stimulation in patients exposed to chemotherapy compared with patients who were chemotherapy-naïve. **Methods** A retrospective cohort study of 130 patients with cancer or autoimmune disease was performed. Demographics, ovarian reserve, ovarian response and stimulation parameters, and oocyte data were compared between patients who were pre- and post-chemotherapy. Logistic regression modeling was performed to identify risk factors for cancellation and low oocyte yield, adjusting for confounders as appropriate. **Results** Antral follicle count (AFC) was significantly lower in post-chemo patients (9 vs. 17, $p<0.001$). Post-chemotherapy patients were more likely to be cancelled during stimulation (23 vs. 4 %, $p=0.003$). Among those that went to retrieval, there was no difference in total number of oocytes (10 vs. 10, $p=0.31$) or mature oocytes retrieved (8 vs. 8, $p=0.38$), despite higher

starting (300 vs. 450 IU, $p<0.001$) and total gonadotropin (3075 vs. 4612.5 IU, $p=0.008$) doses in post-chemotherapy patients. Low AFC (≤ 6) was associated with cycle cancellation (OR 7.7, 95 % CI 1.8–33.2) and low oocyte yield (<6) (OR 5.4, 95 % CI 1.6–17.7).

Conclusions Patients post-chemotherapy have lower AFC compared with the chemotherapy-naïve and have higher cancellation rates. Among those who underwent oocyte retrieval, oocyte yield was similar in both groups. Low AFC was most strongly associated with cycle cancellation and oocyte yield. Post-chemotherapy patients had higher rates of cycle cancellation but did equally well as pre-chemotherapy patients if they reached retrieval.

Keywords Fertility preservation · Cancer · Chemotherapy · Ovarian stimulation

Capsule Chemotherapy has a known gonadotoxic effect, however the specific impact on ovarian stimulation parameters/outcomes is unclear. Though post-chemotherapy patients had lower AFC and higher risk of cancellation, those who reached retrieval had similar oocyte yield as those who were pre-chemotherapy. It is reasonable to offer fertility preservation/treatment after chemotherapy with individualized protocols.

✉ Jessica L. Chan
jessica.chan@uphs.upenn.edu

¹ Department of Obstetrics and Gynecology, University of Pennsylvania, 3701 Market Street, Suite 800, Philadelphia, PA 19104, USA

² Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, 423 Guardian Drive, 605 Blockley Hall, Philadelphia, PA 19104, USA

Introduction

Chemotherapy is often the first-line treatment for both cancer and benign autoimmune disease. While many of these drugs are curative and life saving, many types of chemotherapy are known to be gonadotoxic and may have a negative impact on ovarian reserve and future fertility for reproductive-aged women who have received them [1]. Alkylating agents, such as cyclophosphamide, are well known to cause follicular depletion, oocyte destruction, and stromal fibrosis [2–4], and the risk of ovarian dysfunction appears to occur in a dose-dependent fashion [5]. This effect has been demonstrated in both cancer patients [5–8] and in patients with autoimmune disease such as systemic lupus erythematosus and granulomatosis with polyangiitis [9, 10]. Variable toxicity has been documented for multidrug regimens. For example, regimens used to treat Hodgkin's lymphoma such as bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) are associated

with a high incidence of ovarian toxicity, while regimens such as doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) are associated with less risk [11–13].

As chemotherapy has improved survivorship after cancer and other medical illnesses in women of reproductive age, the importance of fertility is clear as these women age and become ready to start a family [14]. Due to ovarian damage from exposure to chemotherapy, these patients are more likely to experience infertility and require fertility treatment in order to conceive [15]. In vitro fertilization (IVF) treatment protocols and fertility preservation cycles in patients treated with gonadotoxic chemotherapy are of interest to clinicians who wish to optimize care for these patients.

Ovarian stimulation has been shown to be impaired in cancer patients even before treatment [16, 17]. Women with cancer have been demonstrated to have a lower oocyte yield and lower estradiol levels during controlled ovarian hyperstimulation [17]. In patients who have undergone chemotherapy, one would expect this impairment to be even more pronounced. Few studies to date have assessed the success of assisted reproductive technologies and fertility preservation in patients who have been exposed to chemotherapy [16, 18, 19]; however, results have been mixed.

The objective of this study was to evaluate the response to ovarian stimulation in patients with a history of cancer or benign disease treated with gonadotoxic chemotherapy as compared with patients who are chemotherapy-naïve. We hypothesize that patients treated with chemotherapy have impaired baseline ovarian reserve markers compared to unexposed patients, and that these patients will respond poorly to ovarian stimulation, with higher rates of cancellation and lower oocyte yield at retrieval.

Materials and methods

Patients with a history of cancer or medical illness requiring treatment with gonadotoxic chemotherapy that underwent ovarian stimulation for oocyte or embryo banking at Penn Fertility Care between 2006 and 2014 were identified in this retrospective cohort study. Institutional Review Board approval was obtained from the University of Pennsylvania. Patients between the ages of 18 and 50 with a diagnosis of cancer or another medical illness requiring gonadotoxic chemotherapy were included in the study. Those who had previously been exposed to chemotherapy were defined as “post-chemotherapy” and those who had not been exposed to chemotherapy prior to ovarian stimulation were defined as “pre-chemotherapy”.

Ovarian stimulation was performed using either luteal-phase GnRH agonist or GnRH antagonist protocols as previously described [20]. The starting dose of gonadotropin dose was selected based on patient age, AMH, and baseline AFC. Most patients with breast and endometrial cancer also received an aromatase inhibitor. Under this protocol, letrozole 5 mg (Mylan Pharmaceuticals or Teva Pharmaceuticals) was administered daily starting the

second or third day of a spontaneous menstrual cycle and continuing until the day of HCG or GnRH agonist administration for final oocyte maturation. Patients were only cancelled for complete lack of response to gonadotropins with no follicular development. This cancellation policy applied to both groups of patients. Patients elected to cryopreserve embryos or oocytes. For embryos, oocytes were fertilized via conventional insemination or ICSI and embryos cryopreserved via slow freeze or vitrification. For oocyte cryopreservation, oocytes were stripped on the day of retrieval to assess maturity prior to cryopreservation via vitrification.

Detailed demographic- and treatment-specific information were obtained from medical records. Demographics details included the following: age at diagnosis, age at time of fertility consultation, race, gravidity, parity, body mass index (BMI), and partner status. Disease-specific information collected included the following: specific medical diagnosis and treatment received prior to consultation. Stimulation data obtained included the following: baseline ovarian reserve testing (baseline follicle-stimulating hormone and estradiol, Anti-Müllerian hormone levels, antral follicle count), stimulation protocol, starting gonadotropin dose, total gonadotropin usage, duration of stimulation, peak estradiol level (pg/mL), oocyte yield (mature and immature), and cycle cancellation.

Statistical analysis

Demographic characteristics were compared between pre- and post-chemotherapy patients using Wilcoxon rank sum test and Student's *t* test as appropriate, Pearson Chi-squared test and Fisher's Exact test, for continuous and categorical variables as appropriate. All hormone concentrations were natural-log transformed to reduce the influence of left-skewed distribution of values. To accommodate multiple, and possibly correlated, cycles per woman, we utilized an extension to logistic regression STATA xtlogit procedure [21]. These models were used to identify risk factors for cycle cancellation and low oocyte yield, adjusting for confounders as appropriate. A two-tailed *p* value of <0.05 was considered statistically significant. Statistical analysis was performed using STATA version 13.0 (StataCorp, College Station, TX, USA).

Results

Patient characteristics

A total of 130 patients met our inclusion criteria; 95 patients were pre-chemotherapy and 35 patients were post-chemotherapy before ovarian stimulation. Demographic characteristics of the cohort are presented in Table 1. Patients who were exposed to chemotherapy were younger both at age of consult (21.7 vs. 31, $p<0.001$) and at the time of fertility preservation consultation (27.7 vs. 32, $p<0.001$). More time had

Table 1 Demographic characteristics by chemotherapy exposure

	Pre-chemotherapy (N=95)	Post-chemotherapy (N=35)	<i>p</i> value*
Age at diagnosis (years)	31 (1–43)	21.7 (8–35)	<0.001
Age at consultation (years)	32 (17–43)	27.7 (17–39)	<0.001
Time elapsed since diagnosis (years)	0.17 (0.002–18.5)	4.5 (0.32–29.1)	<0.001
Race (Caucasian)	77 (81 %)	28 (80 %)	0.88
Partnered	71 (75 %)	24 (69 %)	0.64
Body Mass Index (kg/m ²)	22.0 (16.0–47.4)	22.5 (17.2–33.0)	0.34
Previous pregnancy	35 (37 %)	8 (23 %)	0.13
Diagnosis:			
Breast cancer	53 (57 %)	3 (9 %)	<0.001
Hematologic cancer	14 (15 %)	24 (69 %)	<0.001
Gynecologic cancer	11 (12 %)	1 (3 %)	
Other cancer	7 (8 %)	4 (11 %)	
Benign disease	8 (9 %)	3 (9 %)	
Chemotherapy regimen:			
Alkylating agent	–	24 (69 %)	–
Alkylator score	–	1 (0–7)	–
Time from last chemotherapy (months)	–	18 (0–360)	–
Less than 6 months		11 (31 %)	
Less than 12 months		14 (40 %)	

Values are median (range) or n (%)

* *p* values calculated from Pearson Chi-squared Student's *t* test, Mann Whitney U or Fisher's exact tests as appropriate

elapsed between time of diagnosis and fertility consultation in the post-chemotherapy group (4.5 vs. 0.17 years, $p<0.001$). Patients were similar with respect to race, partner status, BMI, and previous pregnancy history. The majority of patients in the pre-chemotherapy group had a diagnosis of breast cancer (57 %), while the majority of patients in the post-chemotherapy group had a history of hematologic cancer (69 %). More patients in the pre-chemotherapy group (92 %) were about to face impending treatment as compared with the post-chemotherapy group (49 %, $p<0.001$). Benign diseases requiring chemotherapy included microscopic polyangiitis, granulomatosis with polyangiitis, sarcoidosis, mixed connective tissue disorder, sickle cell anemia, lymphangioleiomyomatosis, pigmented villonodular synovitis, and myasthenia gravis. In the post-chemotherapy group, the majority of patients had received an alkylating agent (69 %) with a median cumulative alkylator score of 1 (range 0–7, where a higher score is reflective of a higher exposure and risk for ovarian toxicity) [1]. The median time since last chemotherapy received was 18 months, with ($n=11$) of patients receiving gonadotoxic chemotherapy within 6 months of ovarian stimulation.

Ovarian reserve and protocol characteristics

Ninety-five pre-chemotherapy patients underwent a total of 108 cycles of ovarian stimulation. Thirty-five post-

chemotherapy patients underwent 42 cycles. Table 2 compares baseline ovarian reserve measures, and first-cycle protocol characteristics were compared in pre- and post-chemotherapy patients. Compared to pre-chemotherapy patients, post-chemotherapy patients had no differences in levels of a baseline follicle-stimulating hormone (FSH), estradiol, or anti-Mullerian hormone (AMH). Median baseline antral follicle count (AFC) was significantly lower in post-chemotherapy patients (9 vs. 17, $p<0.001$). As AFC may be lower in patients with recent hormonal contraceptive exposure [22], we restricted the analysis to patients who were not on oral contraceptives at the time of baseline testing, and this difference remained significant (9 vs. 17, $p=0.02$). A gonadotropin-releasing hormone (GnRH) antagonist protocol was used in the majority of patients (93 % of pre-chemotherapy patients and 83 % of post-chemotherapy patients). Fifty-one patients in the pre-chemotherapy group and three in the post-chemotherapy were given an aromatase inhibitor (letrozole 5 mg by mouth daily), starting the second or third day of the cycle and continued until the day of human chorionic gonadotropin (HCG) or GnRH agonist administration. Twelve of the cycles (11 %) in the pre-chemotherapy and one of the cycles (2 %) in the post-chemotherapy groups were luteal phase starts. Post-chemotherapy patients were started at higher doses of gonadotropins compared with pre-chemotherapy patients (450 vs. 300 IU, $p<0.001$).

Table 2 Baseline ovarian reserve measures and first-cycle protocol characteristics by chemotherapy exposure

	Pre-chemotherapy (N=95)	Post-chemotherapy (N=35)	<i>p</i> value*
Baseline estradiol (pg/mL) ^a	51.7 (45.1–59.4)	38.7 (32.6–45.9)	0.03
Baseline FSH (mIU/mL) ^{a, b}	5.0 (4.1–6.0)	5.3 (3.6–7.7)	0.34
Baseline AMH (ng/mL) ^{a, c}	1.4 (1.0–1.9)	0.7 (0.3–1.7)	0.08
Baseline antral follicle count	17 (2–61)	9 (0–37)	<0.001
Oral contraceptive use at baseline	45 (49 %)	13 (37 %)	0.33
Stimulation protocol			
GnRH antagonist	87 (94 %)	30 (86 %)	<0.001
GnRH agonist long protocol	6 (6 %)	5 (14 %)	
Aromatase inhibitor used	45 (48 %)	3 (9 %)	
Starting gonadotropin dose (IU)	300 (100–600)	450 (150–600)	<0.001

Abbreviations: *FSH*, follicle-stimulating hormone, *AMH*, anti-Mullerian hormone

Values are median (range)

* *p* values calculated from Pearson Chi-square, Student's *t* test, Mann Whitney U or Fisher's exact tests as appropriate^a Geometric mean presented (95 % CI)^b FSH available for 79 in post-chemotherapy and 31 in pre-chemotherapy groups^c AMH available for 48 in post-chemotherapy and 14 in pre-chemotherapy groups**Outcomes of ovarian stimulation**

Ovarian stimulation outcomes are compared in Table 3. Post-chemotherapy patients used higher total doses of gonadotropin during stimulation compared with pre-chemotherapy

patients (4612.5 vs. 3075 IU, $0=0.008$). Length of stimulation was similar (11 vs. 11 days, $p=0.78$). Post-chemotherapy patients had a lower total number of follicles (11 vs. 19, $p=0.02$) and fewer follicles measuring >14 mm (5 vs. 11, $p=0.01$) on the day of trigger. Peak estradiol levels were similar between

Table 3 Outcomes of ovarian stimulation by chemotherapy exposure

	Pre-Chemotherapy (N=95)	Post-Chemotherapy (N=35)	<i>p</i> value*
Total gonadotropin dose (IU)	3075 (900–7800)	4612.5 (600–7200)	0.008
Total follicle number on day of trigger	19 (2–63)	11 (0–56)	0.02
Total follicles >14 mm	11 (0–37)	5 (0–22)	0.01
Estradiol at trigger (pg/mL) ^a	929.2 (754.4–1144.6)	947.1 (566.1–1584.3)	0.44
Trigger medication:			
HCG only	61 (67 %)	21 (78 %)	0.51
GnRH agonist only	8 (9 %)	1 (4 %)	
HCG 1000 units+GnRH agonist 80 units (Co-trigger)	22 (24 %)	5 (19 %)	
Days of stimulation	11 (6–23)	11 (2–13)	0.78
Cancellation rate	4 (4 %)	8 (23 %)	0.003
Number of oocytes retrieved	10 (0–41)	10 (2–27)	0.31
Number of mature oocytes	8 (0–34)	8 (2–19)	0.38
Number of immature oocytes	3 (0–17)	2 (0–8)	0.07
Maturation rate	79.2 % (0–100 %)	72.7 % (0.6–100 %)	0.78
Fertilization rate ^b	55.6 % (0–100 %)	58.6 % (0.5–100 %)	0.63

Abbreviations: *HCG*, human chorionic gonadotropin, *GnRH*, gonadotropin-releasing hormoneValues are median (range) or *n* (%)* *p* values calculated from Pearson Chi-square, Student's *t* test, Mann Whitney U or Fisher's exact tests as appropriate^a Geometric mean presented (95 % CI)^b Among patients who created embryos

pre- and post-chemotherapy groups (929.2 vs. 947.1 pg/mL, $p=0.44$), even when excluding cycles where letrozole was used (1589.1 vs. 1012.1 pg/mL, $p=0.45$). The majority of patients received an HCG trigger (pre-chemotherapy 67 %, post-chemotherapy 78 %). There was a higher rate of first-cycle cancellation in the post-chemotherapy group as compared with the pre-chemotherapy group (23 vs. 4 %, $p=0.003$). Patients were only cancelled for complete lack of response to gonadotropins (no follicular development). However, in patients that went to retrieval, there was no difference in number of oocytes retrieved (10 vs. 10, $p=0.31$) or number of mature oocytes retrieved (8 vs. 8, $p=0.38$). A subgroup analysis was performed to determine the effect of recent chemotherapy exposure (<6 months) on first-cycle ovarian stimulation outcomes in the post-chemotherapy group. There was a difference in baseline FSH between those with chemotherapy within 6 months of stimulation (2.6 mIU/mL) and those who had more remote chemotherapy (>6 months) (8.0 mIU/mL, $p=0.02$) and fertilization rate (recent chemo 100 % vs. remote chemo 58.6 %, $p=0.04$). There was no significant difference in baseline AMH or AFC, starting or total dose of gonadotropins used, days of stimulation, cancellation rate, number of eggs retrieved, or number of mature eggs retrieved.

Association with cycle cancellation and low oocyte yield

To determine the factors associated with cycle cancellation, repeated measure logistic regression was performed to account for the number of cycles per woman. We also included covariates for age at the time of consultation and post-chemotherapy status. Women with a baseline AFC of less than or equal to 6 were more likely than women with higher AFC to experience cycle cancellation (adjusted OR 7.7, 95 % CI 1.8–33.2, $p=0.006$) and oocyte yield less than 6 (adjusted OR 5.4, 95 % CI 1.6–17.7, $p=0.005$) (Tables 4 and 5). The relationships remained significant even after controlling for oral contraceptive use (cancellation OR 5.99, 95 % CI 1.25–28.74, low oocyte yield OR 5.14, 95 % CI 1.55–17.0). Fig. 1 demonstrates an inverse dose-response relationship between AFC and the percentage of subjects cancelled. The lower the antral

follicle count, the higher the likelihood of cancellation. When considering AFC as a continuous value, the risk of cancellation decreased by 40 % for each antral follicle seen on baseline ultrasound (adjusted OR 0.6, 95 % CI 0.5–0.9) and the risk of low oocyte yield decreased by 10 % for each antral follicle (adjusted OR 0.9, 95 % CI 0.9–1.0). Baseline AMH was significantly associated with oocyte yield (adjusted OR 0.3, 95 % CI 0.1–0.8), but not with cancellation (adjusted OR 0.2, 95 % CI 0.01–2.8). For every unit increase in AMH, the risk of low egg yield decreased by 70 %. This relationship remained significant even after controlling for oral contraceptive use (OR 0.37, 95 % CI 0.15–0.91). Post-chemotherapy status did not appear to have a significant association with either cancellation (adjusted OR 37.6, 95 % CI 0.9–1551.4, $p=0.056$) or low oocyte yield (adjusted OR 2.7, 95 % CI 0.6–12.9, $p=0.203$).

Discussion

In this study, we demonstrated that despite having lower baseline antral follicle counts and higher rates of cancellation during controlled ovarian stimulation than those who are pre-chemotherapy, post-chemotherapy patients who undergo retrieval have similar oocyte yield and maturation rates as those who are pre-chemotherapy. This is one of the largest studies to examine post-chemotherapy ovarian stimulation in patients with cancer or medical illnesses requiring chemotherapy.

While there were no significant differences in baseline hormonal testing in our study (FSH, estradiol, or AMH), patients treated with chemotherapy had a significantly lower number of antral follicles at baseline pelvic ultrasound compared with those who were chemotherapy-naïve, consistent with findings from previous studies. The gonadotoxic effects of chemotherapy on ovarian reserve markers are well documented. In a population-based study of 100 childhood cancer survivors who had all been exposed to chemotherapy compared with similar-aged controls, survivors were found to have increased rates of premature ovarian failure and lower antral follicle counts [23]. A smaller study of 22 breast cancer patients following chemotherapy demonstrated increased baseline FSH,

Table 4 Associations with cycle cancellation

	Unadjusted OR (95 % CI)	<i>p</i> value	Adjusted OR (95 % CI)*	<i>p</i> value
Post-chemotherapy	20.8 (1.3–339.1)	0.033	37.6 (0.9–1551.4)	0.06
Age at time of consultation	1.0 (0.8–1.2)	0.873	1.1 (0.9–1.4)	0.35
Baseline AMH	0.3 (0.1–0.7)	0.005	0.2 (0.01–2.8)	0.22
Baseline antral follicle count	0.7 (0.5–0.8)	0.002	0.6 (0.5–0.9)	0.003
Baseline antral follicle count <6	10.3 (2.7–39.6)	0.001	7.7 (1.8–33.2)	0.006

Abbreviations: AMH, anti-Müllerian hormone

* Adjusted for age at the time of consultation, cycle number, post-chemotherapy status

Table 5 Associations with low oocyte yield (<6)

	Unadjusted OR (95 % CI)	<i>p</i> value	Adjusted OR (95 % CI)*	<i>p</i> value
Post-chemotherapy	2.1 (0.6–7.4)	0.23	2.7 (0.6–12.9)	0.20
Age at time of consultation	1.1 (1.0–1.2)	0.01	1.2 (1.0–1.3)	0.02
Baseline AMH	0.3 (0.2–0.7)	0.005	0.3 (0.1–0.8)	0.02
Baseline antral follicle count	0.9 (0.8–1.0)	0.004	0.9 (0.9–1.0)	0.02
Baseline antral follicle count <6	6.7 (2.2–20.4)	0.001	5.4 (1.6–17.7)	0.005

Abbreviations: *AMH*, anti-Mullerian hormone

* Adjusted for age at the time of consultation, cycle number, post-chemotherapy status

lower AMH, and antral follicle counts compared with breast cancer patients pre-chemotherapy and with healthy controls [24].

Previous data is mixed with regard to ovarian response in women with malignant disease. While a retrospective study published by Almog et al. demonstrated no difference in the number of dominant follicles or number of oocytes retrieved between pre-treatment cancer patients and age-matched controls during controlled ovarian hyperstimulation for IVF [25], other studies have suggested that ovarian response is poor, even before treatment. A systematic review and meta-analysis published by Friedler et al. reports that women with malignant disease with no previous exposure to chemotherapy should expect lower oocyte yield after controlled ovarian hyperstimulation for fertility preservation, possibly related to the negative effect of the disease state on the hypothalamic-gonadal axis and subsequent response to stimulation [16]. Taking this into account, patients with cancer and illnesses requiring chemotherapy prior to treatment were chosen as a comparison group in this study to evaluate the response to ovarian stimulation in a similar group of patients who were post-treatment. Patients who were post-chemotherapy required both higher starting and total doses of gonadotropins during ovarian stimulation and were more likely to be cancelled during stimulation. However, despite lower baseline

AFC and higher cancellation rates, those who reached oocyte retrieval had similar outcomes as those who were pre-chemotherapy for oocyte yield and maturation rate. Post-chemotherapy patients who reached retrieval had similar oocyte yield, likely due to higher starting and total gonadotropin use. These higher doses were likely used to compensate for the lower baseline AFC in the post-chemotherapy groups. These findings are consistent with those found in a retrospective cohort study of patients who underwent fertility preservation before and after chemotherapy treatment for hematological disorders that reported similar oocyte and embryo yield between groups [18]. Similarly, a study comparing women undergoing in vitro fertilization after systemic cancer treatment with women who received local cancer treatment found that there was no difference in oocyte yield between the groups [26]. In contrast to our findings, a small study by Das et al. of 23 post-chemotherapy patients found that these patients had worse response to gonadotropins and lower oocyte yield at retrieval. However, their comparison group was comprised of healthy, unexposed patients with male factor infertility.

We also demonstrated that a low antral follicle count (<6) was strongly associated with both cycle cancellation and low egg yield. While there is no widely used AFC threshold at which ovarian response is significantly decreased, multiple studies in non-cancer IVF populations have demonstrated a correlation between low antral follicle count and poor response to controlled ovarian hyperstimulation [27–30]. In an analysis of 1,049 stimulated IVF cycles by Hsu et al., AFC was predictive of both cancellation rate and oocytes retrieved [29]. The odds ratio for cancellation with an AFC between 1 and 5 was 8.3 (95 % CI 1.9–35.6) for a long protocol and 7.4 (95 % CI 2.1–26.9) for a microdose GnRH agonist protocol, as compared with a referent group with an AFC of ≥ 16 . The number of oocytes retrieved appeared to also increase with higher AFC. A study in healthy oocyte donors using an AFC cut-off of <10 found similar findings [30]. Though there is concern that pre-treatment oral contraceptive use may be associated with lower antral follicle count [22, 31], low AFC remained significantly associated with both cancellation and low oocyte yield at retrieval even after controlling for oral

Fig. 1 Percent cancellation by the antral follicle count (AFC) group

contraceptive use in multivariable regression. Additionally, an inverse relationship between antral follicle count and percent cancellation was also demonstrated in this study. The lower the antral follicle count, the higher the percent of cancellation (Fig. 1). Given this finding, it is reasonable to regard an AFC of <6 in populations of patients with cancer or medical illnesses requiring chemotherapy as predictive of poor ART outcomes (cancellation or low oocyte yield). Patients with an AFC of <6 require individualization of treatment to maximize response during ovarian stimulation as well as counseling about the possibility of poor response. However, as it appears that post-treatment patients who reach oocyte retrieval have similar outcomes to those who are pre-treatment, they should be encouraged to proceed with fertility preservation, if so desired. Additionally, post-chemotherapy status did not appear to have a significant association with either cancellation (OR 37.6, 95 % CI 0.9–1551.4) or with low oocyte (OR 2.7, 95 % CI 0.6–12.9) yield after adjusting for confounders. However, the effect may still be present but not detected due to the small sample size of the post-chemotherapy group and limited statistical power, as suggested by the large confidence intervals.

While baseline AMH was not significantly different between pre- and post-chemotherapy patients, high AMH was less likely to be associated with low oocyte yield in this study (adjusted OR 0.3, 95 % CI 0.1–0.8). OR of 0.3 can be interpreted as a 70 % reduction in low oocyte yield for each additional unit increase of AMH. This is consistent with prior studies in healthy populations demonstrating the predictive power of AMH for response to stimulation and ART outcomes [32, 33]. Low AMH has been associated with poor response to stimulation and oocyte yield [34–36] and may also be useful information prior to starting the cycle. Whereas prior studies in non-cancer populations have shown an association between low AMH and high cancellation rates [35, 37], this study in patients with cancer or medical conditions requiring chemotherapy did not demonstrate a significant association after adjustment for confounders.

Due to a limited sample size, our study population includes heterogeneous medical conditions, including both patients with cancer and benign diseases who underwent different gonadotoxic treatments. As such, it may be difficult to extrapolate our results for specific diagnoses or treatment regimens and thus may limit generalizability. It is helpful to note that the majority of patients were treated with an alkylating agent (69 %). As our study population represents women who were referred for fertility preservation or treatment consultation, selection bias may exist as the patients included may not be representative of all women with medical diagnoses requiring chemotherapy. Also, due to the retrospective nature of this study, selection bias influencing the types of treatment that the pre- and post-chemotherapy patients received could influence the results. Additionally, retrospective studies are also subject to information bias, as the data was abstracted through

chart review. Moreover, patients received various protocols for ovarian stimulation over the study duration, including letrozole and luteal start protocols. Several studies have demonstrated that such stimulation protocols (letrozole and luteal/random start) do not impact outcomes [38–40], so we do not believe that protocol substantially confounded our results. We examined the effect of letrozole in our multivariable models and found that the use of letrozole in ART protocols was not a confounder of cancellation or low oocyte yield. As there was only one patient in the post-chemotherapy group that underwent a random start, it was not possible to adjust for this factor in our model. Moreover, it was difficult to explore the effect of time since chemotherapy exposure on ovarian stimulation given the heterogeneous nature of the post-treatment population studied.

Few studies to date have compared response to ovarian stimulation between patients who have been treated with gonadotoxic chemotherapy and cancer patients who are chemotherapy-naïve. The current study is one of the largest studies to date and provides insight into the effect of chemotherapy itself on stimulation parameters and outcomes. However, as the majority of the oocytes of the patients in our study remain cryopreserved, we do not have outcome data of pregnancies resulting from the use of these gametes. While the literature suggests that pregnancy outcomes of childhood cancer survivors are similar to those of healthy women [41], knowledge is scarce on the effect that recent chemotherapy exposure has on gametes and offspring outcomes. A prospective, longitudinal study following the use of thawed cryopreserved oocytes, fertilization and pregnancy rates, neonatal and childhood outcomes of the women who freeze their oocytes, and embryos following chemotherapy treatment would be invaluable to understanding the long-term effects of chemotherapy on gametes. Particularly in children and young adults who do not have the opportunity to pursue ovarian stimulation prior to treatment, post-treatment ovarian stimulation and oocyte/embryo cryopreservation may be a strategy to maximize future reproductive capacity in this population.

Conclusions

While it is generally accepted that fertility preservation should be performed before exposure to chemotherapy, this study suggests that even though patients who present after chemotherapy may have lower baseline antral follicle counts and higher rates of cancellation, oocyte yield is comparable to chemotherapy-naïve patients if they reach retrieval. Therefore, it would be reasonable to offer ART to patients who desire fertility preservation or treatment after chemotherapy, as efficacy appears to be similar to those who pursue ART prior to chemotherapy. Low antral follicle count (<6) appears to be significantly associated with cancellation and low oocyte yield

at retrieval, so stimulation protocols and counseling must be individualized for patients who present with low AFC for fertility preservation. Low baseline AMH values may also be considered in pre-stimulation counseling for expected oocyte yield.

Acknowledgments This research was supported by the National Institutes of Health (Grant numbers: R01-HD-062797-05- Dr. Gracia, T32 HD007440- Drs. Chan and Johnson).

Conflicts of interest The authors declare that they have no conflicts of interest.

Statement of human rights and informed consent All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this retrospective cohort study, formal consent is not required.

References

- Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, et al. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. *J Clin Oncol: Off J Am Soc Clin Oncol*. 2009;27(16):2677–85. doi:10.1200/JCO.2008.20.1541.
- Miller 3rd JJ, Williams GF, Leisenring JC. Multiple late complications of therapy with cyclophosphamide, including ovarian destruction. *Am J Med*. 1971;50(4):530–5.
- Nicosia SV, Matus-Ridley M, Meadows AT. Gonadal effects of cancer therapy in girls. *Cancer*. 1985;55(10):2364–72.
- Familiari G, Caggiati A, Nottola SA, Ermini M, Di Benedetto MR, Motta PM. Ultrastructure of human ovarian primordial follicles after combination chemotherapy for Hodgkin's disease. *Human Reprod (Oxford, England)*. 1993;8(12):2080–7.
- Gracia CR, Sammel MD, Freeman E, Prewitt M, Carlson C, Ray A, et al. Impact of cancer therapies on ovarian reserve. *Fertil Steril*. 2012;97(1):134–40. doi:10.1016/j.fertnstert.2011.10.040. e1.
- Hensley ML, Reichman BS. Fertility and pregnancy after adjuvant chemotherapy for breast cancer. *Crit Rev Oncol/Hematol*. 1998;28(2):121–8.
- Wallace WH, Blacklay A, Eiser C, Davies H, Hawkins M, Levitt GA, et al. Developing strategies for long term follow up of survivors of childhood cancer. *BMJ*. 2001;323(7307):271–4.
- Damewood MD, Grochow LB. Prospects for fertility after chemotherapy or radiation for neoplastic disease. *Fertil Steril*. 1986;45(4):443–59.
- Mok CC, Chan PT, To CH. Anti-mullerian hormone and ovarian reserve in systemic lupus erythematosus. *Arthritis Rheum*. 2013;65(1):206–10. doi:10.1002/art.37719.
- Clowse ME, Copland SC, Hsieh TC, Chow SC, Hoffman GS, Merkel PA, et al. Ovarian reserve diminished by oral cyclophosphamide therapy for granulomatosis with polyangiitis (Wegener's). *Arthritis Care Res*. 2011;63(12):1777–81. doi:10.1002/acr.20605.
- Hodgson DC, Pintilie M, Gitterman L, Dewitt B, Buckley CA, Ahmed S, et al. Fertility among female hodgkin lymphoma survivors attempting pregnancy following ABVD chemotherapy. *Hematol Oncol*. 2007;25(1):11–5. doi:10.1002/hon.802.
- Harel S, Ferme C, Poirot C. Management of fertility in patients treated for Hodgkin's lymphoma. *Haematologica*. 2011;96(11):1692–9. doi:10.3324/haematol.2011.045856.
- Blumenfeld Z. Chemotherapy and fertility. *Best Pract Res Clin Obstet Gynaecol*. 2012;26(3):379–90. doi:10.1016/j.bpobgyn.2011.11.008.
- Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. *Cancer*. 2012;118(6):1710–7. doi:10.1002/ncr.26459.
- Barton SE, Najita JS, Ginsburg ES, Leisenring WM, Stovall M, Weathers RE, et al. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. *Lancet Oncol*. 2013;14(9):873–81. doi:10.1016/S1470-2045(13)70251-1.
- Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. *Fertil Steril*. 2012;97(1):125–33. doi:10.1016/j.fertnstert.2011.10.014.
- Domingo J, Guillen V, Ayllon Y, Martinez M, Munoz E, Pellicer A, et al. Ovarian response to controlled ovarian hyperstimulation in cancer patients is diminished even before oncological treatment. *Fertil Steril*. 2012;97(4):930–4. doi:10.1016/j.fertnstert.2012.01.093.
- Senapati S, Morse CB, Sammel MD, Kim J, Mersereau JE, Efymow B, et al. Fertility preservation in patients with haematological disorders: a retrospective cohort study. *Reprod Biomed Online*. 2014;28(1):92–8. doi:10.1016/j.rbmo.2013.07.014.
- Klock SC, Zhang JX, Kazer RR. Fertility preservation for female cancer patients: early clinical experience. *Fertil Steril*. 2010;94(1):149–55. doi:10.1016/j.fertnstert.2009.03.028.
- Johnson LN, Dillon KE, Sammel MD, Efymow BL, Mainigi MA, Dokras A, et al. Response to ovarian stimulation in patients facing gonadotoxic therapy. *Reprod Biomed Online*. 2013;26(4):337–44. doi:10.1016/j.rbmo.2013.01.003.
- Conaway MR. A random effects model for binary data. *Biometrics*. 1990;46(2):317–28. doi:10.2307/2531437.
- Johnson LN, Sammel MD, Dillon KE, Lechtenberg L, Schanne A, Gracia CR. Antimullerian hormone and antral follicle count are lower in female cancer survivors and healthy women taking hormonal contraception. *Fertil Steril*. 2014;102(3):774–81. doi:10.1016/j.fertnstert.2014.05.026. e3.
- Larsen EC, Muller J, Schmiegelow K, Reznitzer C, Andersen AN. Reduced ovarian function in long-term survivors of radiation- and chemotherapy-treated childhood cancer. *J Clin Endocrinol Metab*. 2003;88(11):5307–14. doi:10.1210/jc.2003-030352.
- Lutchman Singh K, Muttukrishna S, Stein RC, McGarrigle HH, Patel A, Parikh B, et al. Predictors of ovarian reserve in young women with breast cancer. *Br J Cancer*. 2007;96(12):1808–16. doi:10.1038/sj.bjc.6603814.
- Almog B, Azem F, Gordon D, Pauzner D, Amit A, Barkan G, et al. Effects of cancer on ovarian response in controlled ovarian stimulation for fertility preservation. *Fertil Steril*. 2012;98(4):957–60. doi:10.1016/j.fertnstert.2012.06.007.
- Ginsburg ES, Yanushpolsky EH, Jackson KV. In vitro fertilization for cancer patients and survivors. *Fertil Steril*. 2001;75(4):705–10.
- Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH. Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril*. 1998;69(3):505–10.
- Frattarelli JL, Levi AJ, Miller BT, Segars JH. A prospective assessment of the predictive value of basal antral follicles in in vitro fertilization cycles. *Fertil Steril*. 2003;80(2):350–5.
- Hsu A, Amy M, Knee AB, Bell C, Cook E, Novak AL, et al. Antral follicle count in clinical practice: analyzing clinical relevance. *Fertil Steril*. 2011;95(2):474–9. doi:10.1016/j.fertnstert.2010.03.023.

30. Melo MA, Garrido N, Alvarez C, Bellver J, Meseguer M, Pellicer A, et al. Antral follicle count (AFC) can be used in the prediction of ovarian response but cannot predict the oocyte/embryo quality or the in vitro fertilization outcome in an egg donation program. *Fertil Steril*. 2009;91(1):148–56. doi:[10.1016/j.fertnstert.2007.11.042](https://doi.org/10.1016/j.fertnstert.2007.11.042).
31. Bentzen JG, Forman JL, Pinborg A, Lidegaard O, Larsen EC, Friis-Hansen L, et al. Ovarian reserve parameters: a comparison between users and non-users of hormonal contraception. *Reprod Biomed Online*. 2012;25(6):612–9. doi:[10.1016/j.rbmo.2012.09.001](https://doi.org/10.1016/j.rbmo.2012.09.001).
32. Reichman DE, Goldschlag D, Rosenwaks Z. Value of antimullerian hormone as a prognostic indicator of in vitro fertilization outcome. *Fertil Steril*. 2014;101(4):1012–8.e1. doi:[10.1016/j.fertnstert.2013.12.039](https://doi.org/10.1016/j.fertnstert.2013.12.039).
33. Arce JC, La Marca A, Mimer Klein B, Nyboe Andersen A, Fleming R. Antimullerian hormone in gonadotropin releasing-hormone antagonist cycles: prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. *Fertil Steril*. 2013;99(6):1644–53. doi:[10.1016/j.fertnstert.2012.12.048](https://doi.org/10.1016/j.fertnstert.2012.12.048).
34. Lekamge DN, Barry M, Kolo M, Lane M, Gilchrist RB, Tremellen KP. Anti-Mullerian hormone as a predictor of IVF outcome. *Reprod Biomed Online*. 2007;14(5):602–10.
35. La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update*. 2010;16(2):113–30. doi:[10.1093/humupd/dmp036](https://doi.org/10.1093/humupd/dmp036).
36. Jayaprakasan K, Campbell B, Hopkisson J, Johnson I, Raine-Fenning N. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. *Fertil Steril*. 2010;93(3):855–64. doi:[10.1016/j.fertnstert.2008.10.042](https://doi.org/10.1016/j.fertnstert.2008.10.042).
37. Lee RK, Wu FS, Lin MH, Lin SY, Hwu YM. The predictability of serum anti-Mullerian level in IVF/ICSI outcomes for patients of advanced reproductive age. *Reprod Biol Endocrinol : RB&E*. 2011;9:115. doi:[10.1186/1477-7827-9-115](https://doi.org/10.1186/1477-7827-9-115).
38. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. *Fertil Steril*. 2013;100(6):1673–80. doi:[10.1016/j.fertnstert.2013.07.1992](https://doi.org/10.1016/j.fertnstert.2013.07.1992).
39. von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. *Fertil Steril*. 2009;92(4):1360–5. doi:[10.1016/j.fertnstert.2008.08.011](https://doi.org/10.1016/j.fertnstert.2008.08.011).
40. Sonmezer M, Turkuoglu I, Coskun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. *Fertil Steril*. 2011;95(6):2125 e9–11. doi:[10.1016/j.fertnstert.2011.01.030](https://doi.org/10.1016/j.fertnstert.2011.01.030).
41. Green DM, Sklar CA, Boice Jr JD, Mulvihill JJ, Whitton JA, Stovall M, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. *J Clin Oncol: Off J Am Soc Clin Oncol*. 2009;27(14):2374–81. doi:[10.1200/jco.2008.21.1839](https://doi.org/10.1200/jco.2008.21.1839).