Impact of cancer therapies on ovarian reserve

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Objective: To determine whether measures of ovarian reserve differ between females exposed to cancer therapies in a dose-dependent manner as compared with healthy controls of similar age and late reproductive age.
Design: Cross-sectional analysis of data from a prospective cohort study.
Setting: University medical center.
Patient(s): Seventy-one cancer survivors aged 15–39 years; 67 healthy, similarly aged unexposed subjects; and 69 regularly menstruating women of late reproductive age (40–52 years).
Intervention(s): None.
Main Outcome Measure(s): Early follicular-phase hormones (FSH, E_2, inhibin B, antimüllerian hormone [AMH]) and ovarian ultrasound measurements (ovarian volume and antral follicle counts [AFC]) were compared using multivariable linear regression.
Result(s): In adjusted models, FSH, AMH, and AFC differed between exposed vs. unexposed subjects (FSH 11.12 mIU/mL vs. 7.25 mIU/mL; AMH 0.81 ng/mL vs. 2.85 ng/mL; AFC 14.55 vs. 27.20). In participants with an FSH <10 mIU/mL, survivors had lower levels of AMH and AFC compared with controls. Alkylating agent dose score was associated with increased levels of FSH and decreased levels of AMH. Exposure to pelvic radiation was associated with impairment in FSH, AMH, AFC, and ovarian volume. Antimüllerian hormone was similar in women previously exposed to high-dose cancer therapy and 40–42-year-old controls.
Conclusion(s): Measures of ovarian reserve are impaired in a dose-dependent manner among cancer survivors compared with unexposed females of similar age. Reproductive hormone levels in menstruating survivors exposed to high-dose therapy are similar to those in late-reproductive-age women. The predictive value of measures for pregnancy and menopause must be studied.
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Key Words: Ovarian reserve, antimüllerian hormone, inhibin, cancer, fertility, oncofertility

Although advancements in cancer therapies have led to improvements in long-term survival (1, 2), treatments often lead to infertility and premature ovarian failure (3). The risk of ovarian failure seems to be dependent on the dose of alkylating agent and pelvic radiotherapy received (4–9). However, it is difficult to predict the extent to which reproductive dysfunction will occur. Limited data exist assessing the utility of hormone and ultrasound measures of ovarian reserve in cancer survivors (10–12), and no study has made correlates to the physiologic changes that occur with natural reproductive aging (13–19). Early detection and a better understanding of ovarian function in cancer survivors would facilitate patient counseling about reproductive risks and fertility options.

The aim of this study was to compare measures of ovarian reserve in young cancer survivors with those in unexposed females of similar age, and with a cohort of late-reproductive-age women. We hypothesized that ovarian reserve is impaired in a dose-dependent fashion in subjects exposed to cancer therapies compared with similarly aged unexposed participants, and that subjects exposed to high-dose treatment have measures that approximate those of late-reproductive-age women.

MATERIALS AND METHODS

This study is part of an ongoing prospective cohort study at the University of Pennsylvania (Penn) comparing annual measures of ovarian reserve between females exposed to chemotherapy and similarly aged healthy unexposed females. This report compares measures of ovarian reserve from the first assessment

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with a population-based cohort of late-reproductive-age women (14).

Subjects

Reproductive-age cancer survivors were principally recruited from the Children's Hospital of Philadelphia Survivorship Program and the Transition Program at Penn's Living Well After Cancer Survivorship Program during the years 2006–2010. Inclusion criteria were [1] chemotherapy treatment, [2] at least 1 year from cancer treatment with no evidence of disease, [3] age 15–39 years, [4] postmenarchal, and [5] presence of uterus and both ovaries. Exclusion criteria included history of a brain or ovarian tumor, pregnancy or lactation within 3 months, hormonal contraception or hormone therapy within 4 weeks, and any medical condition other than cancer associated with ovarian dysfunction.

Unexposed controls of similar age to cancer survivors (reproductive age) were identified through health practices affiliated with Penn and advertising. Controls were postmenarchal, with regular menstrual cycles (21–35 days), a uterus, and both ovaries. Exclusion criteria were the same as for survivors.

The institutional review board at the University of Pennsylvania approved this study, and informed consent was obtained from all participants. Study visits occurred on days 1–4 of the menstrual cycle. Subjects stopped exogenous hormones for at least 4 weeks and were seen during the subsequent menstrual cycle. Cancer survivors with irregular cycles or no menses for 6 weeks after stopping hormones were seen any time.

Questionnaires

During a structured interview, detailed information was collected regarding demographics, medical history, menstrual characteristics, pregnancies, infertility history, contraception, medications, and substance use.

Menstrual Data

Subjects were given a menstrual diary and provided the dates of the two most recent menstrual cycles during the interview. Cycle length was calculated as the interval between the two most recent menstrual cycles. Women were categorized as having regular menstrual cycles if they reported regular menses (21–35 days) and no hormone use the previous year.

Physical Examination

Height and weight were measured for calculating body mass index (BMI).

Pelvic Ultrasonography

Uterine volume, ovarian volume, and antral follicle counts (AFC) were determined by ultrasonography. Measurements were performed using a Siemens Sonoline G50 machine, 6.8-MHz probe. Transvaginal ultrasonography was preferred, though transabdominal ultrasound was performed in participants uncomfortable with the transvaginal approach. Uterine and ovarian volumes were calculated using the ellipse formula ($A \times B \times C \times 0.5233$). Antral follicle count was determined for subjects undergoing transvaginal ultrasonography when both ovaries were visualized and was defined as the number of follicles between 2 and 10 mm in average diameter. Numbers of small (2–5-mm follicles) and large antral follicles ($\geq$5–10 mm) were recorded.

Hormone Analysis

Serum hormones were measured at Penn's Clinical Translational Research Center using FSH and E_2 Coat-A-Count kits (Diagnostic Products Corporation) and inhibin B and antimüllerian hormone (AMH) ELISA kits (Diagnostic Systems). The FSH immunoradiometric assay's range is 1.5–100 mIU/mL, with a sensitivity of 0.7 mIU/mL and inter- and intra-assay coefficients of variation (cov) $<$6% and 4%, respectively. The E_2 RIA's range is 20–3,600 pg/mL, with a sensitivity of 7 pg/mL and inter- and intra-assay cov $<$8.1% and 7%, respectively. The inhibin B ELISA's range is 10–531 pg/mL, with a sensitivity of 7 pg/mL and inter- and intra-assay cov $<$8% and $<$6%, respectively. The AMH ELISA's range is 0.050–10.0 ng/mL, with a sensitivity of 0.025 ng/mL and inter- and intra-assay cov $<$8% and 5%, respectively.

Cancer Therapy

Exposure data were obtained by abstracting medical records. Treatment was summarized for chemotherapeutic type, duration, cumulative dose; radiation dose and location; type of bone marrow transplantation (BMT); and surgery. Chemotherapies categorized as alkylating agents included carmustine, busulfan, lomustine, chlorambucil, cyclophosphamide, ifosfamide, melphalan, nitrogen mustard, procarbazine, and thiotepa. Alkylating agent dose scores (AAD; 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. We utilized previously published cutoff points to define the scores (20). Exposure to pelvic radiation was defined as exposure to direct pelvic radiation or total body irradiation (TBI).

To compare reproductive hormone measures with those in naturally aging women, late-reproductive-age women were identified from the Penn Ovarian Aging Cohort Study (POAS), a population-based longitudinal study of reproductive hormone levels over the menopausal transition (21, 22). For selection into the present study, eligibility criteria included age $\geq$40 years, regular menstrual cycles (21–35 days), the presence of a uterus and at least one ovary, and complete early follicular phase reproductive hormone assays performed by the methods described above. Women using hormonal medications or who were pregnant or lactating were excluded.

Data Analysis

An a prior sample size calculation indicated that a total of 120 participants (60 per group) was required for this study. The sample size calculations were based on the published estimated difference in mean FSH and AMH levels between subjects and controls (10), an α of 0.05, power of 0.80, and a 1:1 ratio of exposed to unexposed.

TABLE 1

Baseline characteristics.

Characteristic	Exposed (n = 71)	Unexposed (n = 67)	*P* value
Age (y), mean (range)	25.67 (24.17–27.17)	27.26 (26.10–28.43)	.10
BMI, mean (range)	23.52 (22.46–24.58)	25.14 (23.74–26.53)	.07
Race–Caucasian, % (n)	86 (61/71)	72 (48/67)	<.001
Education >high school, % (n)	86 (61/71)	100 (67/67)	.001
Income (>$60K), % (n)	53 (29/55)	23 (13/56)	<.001
Current smoking, % (n)	3.0 (2/69)	15 (10/65)	<.001
Marital status–single, n (%)	43/66 (65)	46/59 (78)	.11
Previous pregnancy, % (n)	20 (14/71)	31 (20/65)	.14
Previous pregnancies, median (range)[a]	2 (1–3)	1 (1–5)	.64[b]

[a] For participants who had a previous pregnancy.
[b] Kruskal-Wallace rank test.

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Log-transformed hormone levels and ultrasound data were compared using multivariable linear regression models. Subgroup analyses compared measures of ovarian reserve in regularly menstruating women and among exposure groups. Pearson χ^2 analyses or ANOVA and *t* tests were performed for categoric or continuous data, respectively. To correct for multiple comparisons in this analysis, a two-tailed $P<.01$ was considered significant. Statistical analysis was performed using STATA v10.0 (StataCorp).

RESULTS

Seventy-one cancer survivors and 67 similarly aged controls were included. When comparing baseline characteristics of cancer survivors enrolled in this study with those of the overall survivorship cohort (n = 391), mean age of enrolled cancer survivors was slightly higher than those in the survivorship cohort (25.7 vs. 22.8 years, $P=.001$), but the proportion of cancer types was similar between groups. Within the study population, 24 cancer survivors had been treated for lymphoma (15 Hodgkin, 9 non-Hodgkin), 23 leukemia, 10 sarcoma, 4 Wilms' tumor, 3 breast cancer, and 7 other. Of 71 cancer survivors, 63 (89%) received alkylators, 13 (18%) received pelvic radiation, and 16 had a history of BMT (10 with TBI). Median age at diagnosis was 11 years (range, 4 months to 29 years). Thirty-seven (52%) were treated before menarche, and 34 after.

Mean age of participants was 26.4 years (95% confidence interval [CI] 25.5–27.4 years, range 15–39 years.). Mean BMI was 24.3 kg/m^2 (95% CI 23.4–25.2 kg/m^2). Most were Caucasian (83.6%), and 92.8% graduated high school. Comparison of baseline characteristics between exposed and unexposed participants revealed similar age, BMI, and marital status. More exposed subjects were Caucasian, higher in income, and less likely to smoke (Table 1). On average, study visits occurred on cycle day 3 for both groups.

Comparison of menstrual characteristics between groups revealed no difference in age at menarche (12.5 years exposed vs. 12.4 years unexposed, $P=.67$). Ten survivors and four controls stopped exogenous hormones to participate in the study. Ninety-two percent of survivors (65 of 71) and all controls had spontaneous menses while not taking hormones. Sixty-nine percent of survivors (49 of 71) and 94% of controls (63 of 67) reported regular menstrual cycles (21–35 days), with no hormone use during the past year. In those reporting regular menses, cycle length (28.8 days exposed vs. 29.1 days unexposed, $P=.46$) and bleeding length (5.16 days exposed vs. 4.92 days unexposed, $P=.24$) were not different.

Table 2 presents regression models of hormones and ultrasound measures. Unadjusted analyses demonstrated that

TABLE 2

Geometric mean (95% CI) reproductive hormone measures of ovarian reserve in cancer survivors vs. unexposed participants of similar age.

	Unadjusted[a]			Adjusted[b]		
Variable	Exposed (n = 71)	Unexposed (n = 67)	*P* value	Exposed	Unexposed	*P* value
FSH (mIU/mL)	11.35 (9.70–13.28)	7.52 (6.39–8.85)	<.001	11.12 (9.47–13.06)	7.25 (6.00–8.76)	.001
E_2 (pg/mL)	23.89 (20.60–27.70)	39.96 (25.73–34.90)	.037	24.21 (20.88–28.08)	29.41 (24.73–34.97)	.084
Inhibin B (pg/mL)	27.87 (22.42–34.63)	34.32 (27.57–42.72)	.184	27.03 (21.72–33.65)	29.59 (22.99–38.09)	.582
AMH (ng/mL)	0.79 (0.60–1.04)	2.74 (2.07–3.64)	<.001	0.81 (0.61–1.07)	2.85 (2.06–3.96)	<.001
Ovarian volume (mL)	7.19 (6.06–8.32)	9.04 (7.80–10.49)	.045	7.42 (6.13–8.71)	9.29 (7.80–10.79)	.056
AFC[c]	15.0 (11.71–18.35)	26.51 (22.44–30.58)	<.001	14.55 (10.80–18.30)	27.20 (23.05–31.35)	<.001

Note: Geometric mean hormone levels shown.
[a] Unadjusted analysis.
[b] Linear regression model adjusted for mean age, race, and mean BMI.
[c] Antral follicle count results are shown for the 60 exposed and 61 unexposed participants who had transvaginal ultrasound assessment of AFC.

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TABLE 3

Comparison of reproductive hormones in unexposed reproductive-age participants, cancer survivors (low-dose and high-dose), and late-reproductive-age women, restricted to regularly menstruating participants not using hormones over the past year.

Hormone	Unexposed (n = 63)	Low-dose exposure (n = 27)	High-dose exposure (n = 22)	Late-reproductive (n = 69)
FSH (mIU/mL)	6.93 (6.09–7.89)	7.93 (6.63–9.47)	10.60[a] (8.68–12.95)	8.15[b] (7.19–9.23)
E_2 (pg/mL)	31.81 (27.27–37.10)	24.54[b] (19.85–30.34)	22.95[b] (18.10–29.11)	37.45 (32.27–43.47)
Inhibin B (pg/mL)	39.75 (29.88–52.89)	37.90 (25.54–56.23)	30.37 (19.33–47.73)	30.70 (23.40–40.29)
AMH (ng/mL)	3.07 (2.17–4.36)	1.99 (1.23–3.24)	0.52[a] (0.30–0.90)	0.19[a] (0.13–0.26)

Note: Geometric mean (95% CI) hormone levels are shown. Model adjusted for mean BMI and race. High-dose exposure defined as AAD ≥3 or exposure to pelvic radiation including TBI. Low-dose exposure defined as any cancer treatment that does not meet criteria for "high dose exposure."

[a] $P<.001$ vs. reference unexposed group.

[b] $P<.05$ vs. reference unexposed group.

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cancer survivors had significantly higher FSH and lower E_2, AMH, AFC, and ovarian volume compared with unexposed. Models adjusted for age, race, and BMI confirmed statistically significant associations for FSH, AMH, and AFC but not for ovarian volume (Table 2). Adjustment for potential confounders including smoking, income, and education did not change estimates of association and therefore were not included in the models. Antral follicle count <5 mm and AFC ≥5 mm were different between groups ($P<.001$). Overall, AFC correlated with AMH ($r=0.69$, $P=.0001$), and the correlation was similar between small and large AFC. Treatment before or after menarche and time since diagnosis were not associated with outcomes.

When the analysis was restricted to 49 survivors and 63 controls with regular menstrual cycles and no hormone use over the past year, FSH was higher, and E_2, AMH, and AFC were lower in survivors compared with controls (geometric mean hormones: FSH 9.08 vs. 7.09 mIU/mL, $P=.01$; E_2 24.08 vs. 30.28 pg/mL, $P=.046$; AMH 1.13 vs. 2.81 ng/mL, $P<.001$; AFC 16.91 vs. 26.88, $P=.002$). No significant differences were noted in inhibin levels or ovarian volume between groups.

To assess the utility of AMH and AFC in women whose FSH was considered normal, we compared AMH and AFC between 45 survivors and 51 controls with FSH values <10 mIU/mL. In linear regression models adjusted for age, race, and BMI, AMH and AFC were lower in survivors compared with controls (geometric mean AMH: 1.67 vs. 2.96 ng/mL, $P=.004$; AFC: 18.15 vs. 27.40, $P=.006$).

Comparison of measures of ovarian function by AAD in women without a history of pelvic radiation or TBI revealed a dose–response relationship. In models adjusted for age, race, and BMI, for each unit increase in alkylator score, geometric mean FSH values increased by 0.91 mIU/mL ($P=.016$), and geometric mean AMH levels decreased by 0.55 ng/mL ($P=.003$) (Supplemental Fig. 1A and B). Differences in E_2, inhibin B, AFC, and ovarian volume were not statistically significant.

Measures of ovarian reserve were independently associated with exposure to pelvic radiation. Adjusted comparisons of 13 cancer survivors exposed to pelvic radiotherapy (including TBI) with 58 controls revealed that FSH was higher, and AMH, AFC, and ovarian and uterine volumes lower in the survivors compared with controls (geometric mean hormones: FSH 28.41 vs. 9.36 mIU/mL, $P<.001$; AMH 0.15 vs. 1.24 ng/mL, $P<.001$; AFC 2.94 vs. 17.46, $P=.001$; ovarian volume 4.05 vs. 7.97 mL, $P=.01$; uterine volume 30.04 vs. 49.31 mL, $P=.04$).

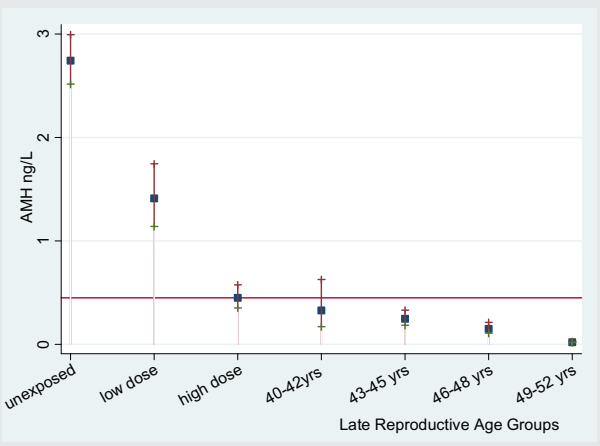
Measures of ovarian reserve were compared between cancer survivors who had undergone BMT with TBI or BMT without TBI and those who had no history of BMT. Compared with survivors without a history of BMT, FSH, E_2, inhibin B, AMH, AFC, and ovarian volume were significantly impaired in survivors with a history of BMT and TBI (geometric mean hormones: FSH 40.42 vs. 9.39 mIU/mL, $P<.001$; E_2 15.09 vs. 25.13 pg/mL, $P=.04$; inhibin B 10.61 vs. 32.92 pg/mL, $P=.003$; AMH 0.01 vs. 1.28 ng/mL, $P<.001$; AFC 0.71 vs. 17.78, $P<.001$; ovarian volume 1.82 vs. 8.21 mL, $P<.001$). Inhibin B levels were also significantly lower in women who had a BMT without TBI compared with the no-BMT group (geometric mean inhibin B 12.95 vs. 32.92 pg/mL, $P=.03$). Six of the 10 subjects with a history of BMT and TBI reported regular menstrual cycles.

Comparison with Late-Reproductive-Age Women

Sixty-nine menstruating late-reproductive-age women from the POAS met inclusion criteria. Except for race, baseline demographic characteristics (age, BMI, employment, education, smoking, and alcohol use) of the 69 eligible participants were no different than the remainder of the original POAS cohort. Although the POAS cohort was designed to be 50% African American, the cohort of cancer survivors was predominately Caucasian. Mean age of the late-reproductive-age women was 45.4 years (range 40–52 years), mean BMI was 28.67 kg/m^2, and 80% of subjects (55 of 69) were Caucasian. Table 3 presents geometric mean early follicular-phase hormone measures in regularly menstruating subjects by group, adjusted for BMI and race. Because of the distribution of treatment, for this analysis, high-dose cancer survivors were defined as having an AAD ≥3 or having been exposed to pelvic radiation including TBI. Overall, FSH was higher and AMH lower in late-reproductive-age women compared with unexposed reproductive-age subjects. Mean levels of AMH in cancer survivors fell between those of unexposed reproductive and unexposed late-reproductive-age groups.

To further compare measures between groups, adjusted geometric mean AMH levels were plotted by group and stratified by age in unexposed late-reproductive-age women (Fig. 1). Antimüllerian hormone levels in the high-dose

FIGURE 1



Geometric mean early follicular phase AMH levels for regularly menstruating participants in each exposure group. Left to right: Mid-reproductive-age unexposed subjects similar in age to cancer survivors; low dose = cancer survivors exposed to low-dose therapy; high dose = cancer survivors exposed to high-dose therapy; and unexposed late-reproductive-aged women, stratified by age. Confidence intervals are shown. High dose defined as AAD ≥3 or exposure to pelvic radiation including TBI. Horizontal reference line represents mean AMH value in cancer survivors exposed to high-dose therapy.

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exposure group were not significantly different from levels in women between the ages of 40 and 42 years. However, levels were significantly lower than in the unexposed and low-dose groups and significantly higher than in the late-reproductive-age group >42 years old ($P<.001$).

DISCUSSION

Recent diagnostic and therapeutic advances in oncology have led to greater survival rates in young cancer patients, but these treatments may deplete the ovarian follicular pool, increasing the risk of ovarian failure and infertility. Although the exact mechanism of ovarian injury is unclear, follicular apoptosis and cortical fibrosis occur (23). In women already treated for cancer, it is difficult to predict the extent to which reproductive dysfunction will occur. Early detection of compromised ovarian function in cancer survivors would be helpful in determining the window of fertility for family planning and anticipating the need for hormone therapy for menopausal symptom management and bone health. Although several hormone and ultrasound measures of ovarian reserve are used to assess natural reproductive aging and predict response and pregnancy in infertile women undergoing assisted reproductive technology, limited data exist assessing the utility of measures of ovarian reserve in cancer survivors (11, 12, 24, 25).

In this study, a comprehensive assessment of ovarian reserve was performed in a population of reproductive-age cancer survivors and similar-age controls. Comparison of measures of ovarian reserve between these groups revealed significant impairment in FSH, AMH, and AFC in cancer survivors. 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 with unexposed females of similar age, supporting subclinical follicular depletion. We also noted a dose-dependent relationship between cancer therapies and measures of ovarian reserve. Specifically, cancer survivors with greater exposure to alkylators, pelvic radiotherapy, or BMT with TBI had the most impaired ovarian reserve. These findings further support the value of these measures to estimate the ovarian follicle pool and hold promise for the assessment of the fertility risk of newly developed therapeutics.

These data corroborate findings from other studies of childhood cancer survivors (10, 25, 26) indicating that FSH, AMH, and AFC are abnormal in cancer survivors. In particular, our data and others suggest that AMH and AFC are very sensitive measures of diminished ovarian reserve in this population. These results differ from a recent report assessing random AMH values in a cohort of 185 survivors, in which significant differences were not detected between survivors and controls. We suspect that no association was detected in that study because of the wide age range and disparity between groups. In addition, our findings with respect to inhibin B are more consistent with those of van Beek et al., who reported that although inhibin B levels were lower in survivors compared with controls, differences did not reach statistical significance (12, 25). Similarly, our findings and others suggest that AMH is a particularly attractive measure of ovarian reserve because levels reflect the number of preantral follicles, fluctuate minimally during the menstrual cycle (27, 28), and do not seem to be influenced by exogenous hormones (29). Previous reports indicate that AMH levels decline with age, predict time to menopause, predict pregnancy after IVF, and are associated with fecundity in the general population (30–36).

To better characterize the degree of oocyte depletion and estimate the "reproductive age" of cancer survivors, hormone measures were compared with those of a cohort of regularly menstruating late-reproductive-age women. Mean levels of AMH in cancer survivors fell between those of unexposed mid-reproductive-age women and unexposed late-reproductive-age women. Interestingly, in stratified analyses, mid-reproductive-age cancer survivors who received highly gonadotoxic therapy had AMH levels similar to those in women 40–42 years of age. The data presented in this report are insufficient to assess the predictive value of ovarian reserve measures or estimate the "reproductive window," and therefore these tests should be interpreted with caution by clinicians. Hormone levels should not be used to predict spontaneous pregnancy rates or need for contraception in cancer survivors (37). However, because the reproductive window cannot be determined, appropriate candidates for pregnancy should not delay childbearing unnecessarily.

The ability to lead full reproductive lives is very important to cancer survivors, and reproductive problems can lead to substantial anxiety and negatively impact quality of life (38, 39). Measures of ovarian reserve may be useful to predict the reproductive impact of cancer therapies so that fertility preservation strategies such as embryo, oocyte, and ovarian tissue cryopreservation can be targeted to those at highest risk. In addition, establishment of an effective

surveillance protocol for the early detection of compromised ovarian function after cancer may allow young cancer survivors to pursue aggressive fertility treatments when there is still a reasonable chance of success.

This study has several strengths. Recall bias was minimized by prospective enrolment, and valid comparisons were made with an unexposed control population of similar age. Confounding has been reduced by restricting the study to nonpregnant, nonlactating females not using hormones and without other causes of ovarian dysfunction. Unlike some studies, a comprehensive evaluation of ovarian reserve was performed and hormone variability minimized by obtaining early follicular phase measures. Cancer diagnoses and treatments were validated with medical records to diminish misclassification bias.

Several limitations should be mentioned. This report represents a cross-sectional analysis of data that cannot prove causation or assess the predictive value of measures for pregnancy and menopause. Although differences in baseline characteristics of unexposed and exposed groups may indicate selection bias, this is unlikely because associations persisted in adjusted analyses. A washout period of 4 weeks after stopping exogenous hormones may be insufficient for normalization of reproductive hormones and may have biased the overall analysis (40). However, significant associations persisted when the analysis was restricted to regularly menstruating subjects who had not been taking hormones over the past year, suggesting that hormone use was not a confounder. Typical of studies involving childhood and young adult cancer survivors, subjects with a variety of diagnoses and treatments were included. Therefore, it was not possible to compare the effect of specific chemotherapeutic regimens on ovarian reserve in this study. Finally, this cohort may not be representative of the general population of survivors. It is possible that participating subjects were more concerned about their fertility than nonparticipants.

Overall, measures of ovarian reserve differ between female cancer survivors and controls in a dose-dependent manner, even if menstrual function is normal. Antimüllerian hormone and AFC seem to be the most sensitive measures of ovarian reserve in this population and are helpful measures for quantifying the damage to the ovaries after cancer therapy. Longitudinal studies of cancer survivors are needed to determine whether impairment in these measures truly reflects fertility and/or predicts time to menopause in cancer survivors.

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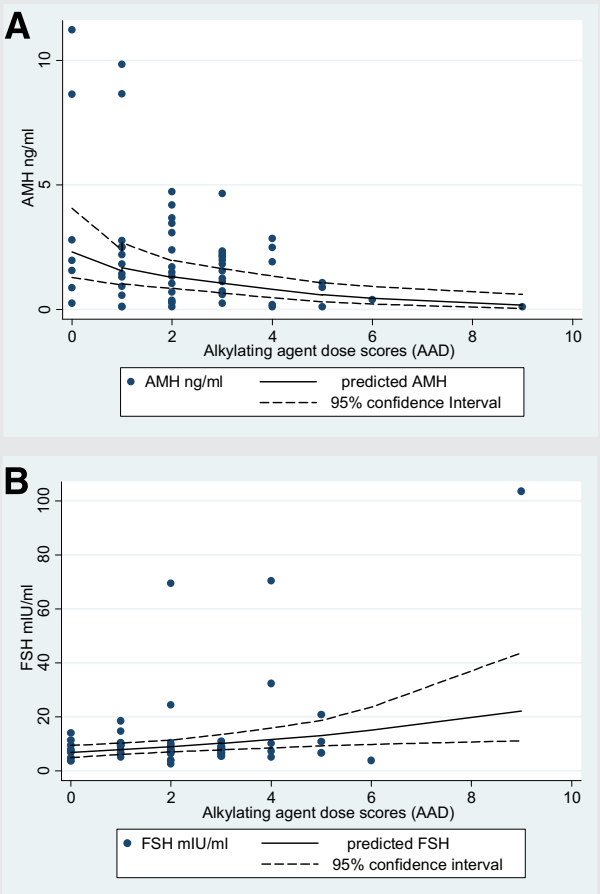
SUPPLMENTAL FIGURE 1



(A) Antimüllerian hormone levels by AAD in women without pelvic radiation. (B) Follicle-stimulating hormone levels by AAD in women without pelvic radiation. Both panels include predicted values for women aged 26.4 years (the average) from the linear regression models along with 95% confidence intervals.

Gracia. Ovarian reserve after cancer. Fertil Steril 2012.