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CLINICAL TRIAL

Impact of adjuvant chemotherapy or tamoxifen-alone on the ovarian reserve of young women with breast cancer

Shari B. Goldfarb[1,2] · Volkan Turan[3,4] · Giuliano Bedoschi[3,5] · Enes Taylan[3] · Nadia Abdo[1] · Tessa Cigler[2] · Heejung Bang[6] · Sujita Patil[1] · Maura N. Dickler[1] · Kutluk H. Oktay[3]

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Abstract

Purpose To determine the longitudinal impact of adjuvant chemotherapy and tamoxifen-only treatments on the reproductive potential of women with breast cancer by using a sensitive ovarian reserve marker anti-Mullerian hormone (AMH) as a surrogate.

Methods One-hundred-and-forty-two women with a primary diagnosis of breast cancer were prospectively followed with serum AMH assessments before the initiation, and 12, 18 and 24 months after the completion of adjuvant chemotherapy or the start of tamoxifen-only treatment. The chemotherapy regimens were classified into Anthracycline-Cyclophosphamide-based (AC-based) and Cyclophosphamide-Methotrexate + 5-Fluorouracil (CMF). Longitudinal data were analyzed by mixed effects model for treatment effects over time, adjusting for baseline age and BMI.

Results Both chemotherapy regimens resulted in significant decline in ovarian reserve compared to the tamoxifen-only treatment ($p < 0.0001$ either regimen vs. tamoxifen for overall trend). AMH levels sharply declined at 12 months but did not show a significant recovery from 12 to 18 and 18 to 24 months after the completion of AC-based or CMF regimens. The degree of decline did not differ between the two chemotherapy groups ($p = 0.53$). In contrast, tamoxifen-only treatment did not significantly alter the age-adjusted serum AMH levels over the 24-month follow up. Likewise, the use of adjuvant tamoxifen following AC-based regimens did not affect AMH recovery.

Conclusions Both AC-based regimens and CMF significantly compromise ovarian reserve, without a recovery beyond 12 months post-chemotherapy. In contrast, tamoxifen-only treatment does not seem to alter ovarian reserve. These data indicate that the commonly used chemotherapy regimens but not the hormonal therapy compromise future reproductive potential.

Keywords Drug therapy · Breast neoplasms · Anti-mullerian hormone · Fertility preservation · Ovarian reserve

Introduction

Breast cancer is the most common malignancy among premenopausal women, with more than 25,000 cases diagnosed annually in women under 45 years of age in the US alone [1, 2]. As societal shifts have led to increased rates

Shari B. Goldfarb, Volkan Turan, and Giuliano Bedoschi have contributed equally to this work.

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✉ Kutluk H. Oktay
correspondence@fertilitypreservation.org;
info@fertilitypreservation.org

[1] Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2] Weill Cornell Medical Center, New York, NY, USA

[3] Department of Obstetrics and Gynecology and Reproductive Sciences, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

[4] Department of Obstetrics and Gynecology, Health and Technology University School of Medicine, Istanbul, Turkey

[5] Department of Obstetrics and Gynecology, Ribeirao Preto School of Medicine, University of Sao Paulo, Ribeirao Preto, Brazil

[6] Division of Biostatistics, Department of Public Health Sciences, University of California, Davis, CA, USA

of women delaying childbearing, a diagnosis of breast cancer is increasingly more likely to occur prior to the completion of family building. Recent advancements in systemic therapies have drastically improved 5-year relative survival rates, which now approach 90% [2]. This improvement in prognosis has allowed providers to dedicate more attention to optimizing patient quality of life, including reproductive function.

Chemotherapy remains the backbone of multimodality therapy for many young breast cancer patients; however, its indisputable survival benefits often come at the expense of fertility. By inducing DNA damage and apoptosis in primordial follicles and potential microvascular and stromal damage in the ovary, cytotoxic chemotherapeutic agents deplete ovarian reserve and compromise ovarian function and fecundability [3–5]. Different chemotherapy regimens confer varying degrees of gonadotoxicity with concomitant rates of decline in ovarian function. The induction of amenorrhea appear to be related to chemotherapy dose and patient age [6]. Additionally, with impaired DNA double stranded break repair mechanisms, BRCA carriers are particularly vulnerable to the gonadotoxic effects of chemotherapy and may experience even more profound declines in ovarian function [7, 8].

Though menstrual history has been used as a surrogate for reproductive potential with evidence of improved fecundability in patients who resume menses following cessation of chemotherapy, the ability to estimate the extent of chemotherapy-induced gonadotoxicity with such indirect surrogate for any given patient remains elusive. Quantifying reproductive potential remains a challenge with a growing body of evidence supporting the use of anti-Mullerian hormone (AMH) as a biomarker of ovarian reserve [9–14]. A glycoprotein and member of the transforming growth factor-B superfamily, AMH is secreted exclusively by granulosa cells of preantral and early antral follicles [15, 16]. AMH has been implicated in the modulation of ovarian follicle development, both presumably suppressing recruitment of primordial follicles as well as attenuating antral follicle response to follicle-stimulating hormone to establish a dominant follicle [17, 18]. The population of developing follicles proportionally reflects the size of the primordial follicle population, thus designating AMH an indirect marker of the primordial follicle reserve and ovarian function [12].

AMH has been used as a predictor for response to ovarian stimulation, as well as a marker of reproductive lifespan [19–24]. It has also emerged as a tool to predict development of chemotherapy-induced amenorrhea and estimate reproductive potential after chemotherapy [25–29]. Declining AMH levels during chemotherapy administration coupled with sustained depressions following completion of chemotherapy have been previously observed in short-term studies, reflecting depletion of the primordial follicle pool [25, 27–29].

However, there is a paucity of longitudinal data defining the long-term trends in AMH levels in breast cancer patients treated with chemotherapy. To be able to quantify the potential impact of chemotherapy administration on an individual patient's reproductive potential would be an invaluable tool to guide accurate patient counseling and decision-making regarding fertility preservation [30]. As chemotherapeutic agents damage both primordial follicles and AMH-producing developing follicles, AMH levels show a sharp decline immediately after treatments. However, if there is remaining primordial follicles, they will give rise to newly developing follicles which will eventually produce AMH, resulting in a "recovery" in levels. Understanding these recovery patterns will also help counsel women and couples on their fertility potential post chemotherapy. Furthermore, the impact of tamoxifen treatment on serum AMH levels is also unknown. Though tamoxifen is not a cytotoxic drug, it is an ovarian-stimulant and can alter ovarian follicle dynamics and hence serum AMH levels.

Therefore our a priori hypothesis was the anthracycline and cyclophosphamide based (AC-based) and CMF regimens but not the tamoxifen-alone treatment will result in significant decline in ovarian reserve. Secondarily, based on the ovarian follicle and AMH production physiology, we hypothesized that the major and clinically meaningful recovery of ovarian reserve from chemotherapy would be at 12 months post-chemotherapy. To that end, we report AMH levels in premenopausal breast cancer patients before, during, and after treatment with chemotherapy or tamoxifen-alone.

Methods

Patient selection

This study was approved by the Institutional Review Boards at all participating institutions (*clinicaltrials.gov* identifier NCT00823654). Enrollment began in January 2009, as part of an NIH-funded translational research project (NICHD and NCI; RO1 HD 053112) to assess the impact of breast cancer chemotherapy on ovarian reserve and ended in November 2017. Women who had prior chemotherapy or ovarian surgery (or those with a planned surgery within a year) and those who did not have regular periods (have more than one irregular cycle-early or late- and/or < 10 spontaneous cycles within the past year) were excluded. Prior known infertility, family history of a first-degree relative with non-surgical menopause at < age 40 years, use of ovarian stimulants within the past 2 months and current pregnancy were also among the exclusion criteria. We also excluded those that

were at age 45 or older as they may be approaching menopause during follow-up and women who had a recurrence and needed additional chemotherapy. Participants provided blood samples prior to chemotherapy and at 12-, 18- and 24-months post treatment.

In general anthracycline and taxane based regimens are used in patients with more advanced stage disease and in tumors with worse molecular subtypes. Given the young age of the patients in this study, more of them were treated with ddAC-T than CMF (Cyclophosphamide-Methotrexate + 5-Fluorouracil) since the biology of their disease was worse. CMF was reserved for patients with smaller, node negative tumors where fertility preservation was of lesser concern (based on the earlier menstruation-based data) [31]. Tamoxifen was given to patients with early stage hormone receptor positive breast cancer who had low Oncotype scores and more favorable prognoses.

Specifics of the serum AMH analysis and the assay

Resulting sera were aliquoted and stored first at – 80 °C then long term at – 273 °C. Frozen aliquots were transported on dry ice to Webster, TX, where serum AMH was measured on site at Ansh Laboratories by one of the team members using an ultra-senstive two-site enzyme-linked immunoassay (picoAMH ELISA, Ansh Labs, Webster, TX) following the manufacturer's instructions. AMH levels were expressed in ng/mL and all assays were performed within three days in mid-August 2017 with a single lot of reagents. Samples were initially diluted 1:10. The reportable range was 0.003 to 23 ng/mL. Initial values falling below 0.03 ng/mL were retested without dilution and any samples with initial values > 11.5 ng/mL were retested at a 1:20 dilution. All final optical densities fell within the standard curve. The coefficient of variation for the four-levels of pooled serum quality controls tested along with study specimens were all < 7%.

Statistical analyses

A priori power calculations originally done in study planning to detect at least 0.18 ng/mL reduction (approximately a 10% drop based on the pilot baseline AMH data before the study inception) in serum AMH levels at the 12-month time point with 80% power and type I error of 0.01 (Type I error is < 0.05 to account for multiple comparisons) compared to baseline with repeated measures of ANOVA. Based on the distribution of treatments at the time of the study planning, we anticipated > 50% of women to receive AC-based treatments with CMF and tamoxifen-only being evenly distributed. Based on these assumptions, we calculated a sample size of 190 women with breast cancer with about 150 completing follow up and remain evaluable.

A mixed effects regression model was fitted with time, treatment group and time-and-treatment interaction for longitudinal data, adjusting baseline age (Fig. S1) and body mass index that can influence AMH levels, where random intercept was used to capture within-person correlation [32]. Regression and inference were performed on the logarithmically transformed (log10) AMH data, while untransformed (raw) data were used for summary statistics and graphs. AMH levels are dependent on the woman's age. A (univariate) generalized linear model was fitted on the referent population (e.g., tamoxifen group with baseline data only) to model AMH levels as a function of age and to derive 'expected' value of AMH at given age (Fig. S2). Statistical analyses were performed with SAS 9.4 (SAS Institute, Cary NC).

Results

Description of the study population and the correlation of baseline AMH with age

Out of the 207 women with breast cancer diagnosis who were enrolled initially, 30 were excluded later because they lacked a baseline AMH and 22 were excluded because they lacked a follow up AMH assessment. Of the 155 women, 13 were excluded for receiving other chemotherapy regimens (cyclophosphamide and docetaxel $n = 8$; vinorelbine, $n = 1$; trastuzumab and paclitaxel; $n = 4$). Of the 142 remaining evaluable women, 106 received AC-based regimens: doxorubicin, cyclophosphamide and paclitaxel ($n = 95$); epirubicin, cyclophosphamide and paclitaxel ($n = 4$); doxorubicin, cyclophosphamide and eribulin ($n = 4$); doxorubicin, cyclophosphamide and nab = paclitaxel ($n = 2$); doxorubicin, cyclophosphamide and lapatinib ($n = 1$). Of the remaining, 19 received CMF (Cyclophosphamide, Methotrexate, 5-Florouracil) while 17 received tamoxifen therapy alone (Fig. 1). Characteristics of those 142 patients are summarized in Table 1. Of those, 80 women completed all three follow ups, 126 completed at least two and 142 completed at least one follow up (Fig. 1). The most frequently completed follow up was at the 12-month time point (127 women). Although women with no follow up AMH levels were excluded from the longitudinal analysis, baseline AMH values from 5 such excluded participants (hence, a total of 17 + 5 = 22 measurements from 22 women) were included to estimate the trend of AMH due to natural aging in the tamoxifen-only group. As expected, in regression analysis, we found the patient age to be an important predictor for baseline AMH levels; higher age at study entry was associated with significantly lower AMH levels. This translated into about 11% decrease in serum AMH levels per increasing year of age before the onset of chemotherapy (using baseline data, $p < 0.0001$).

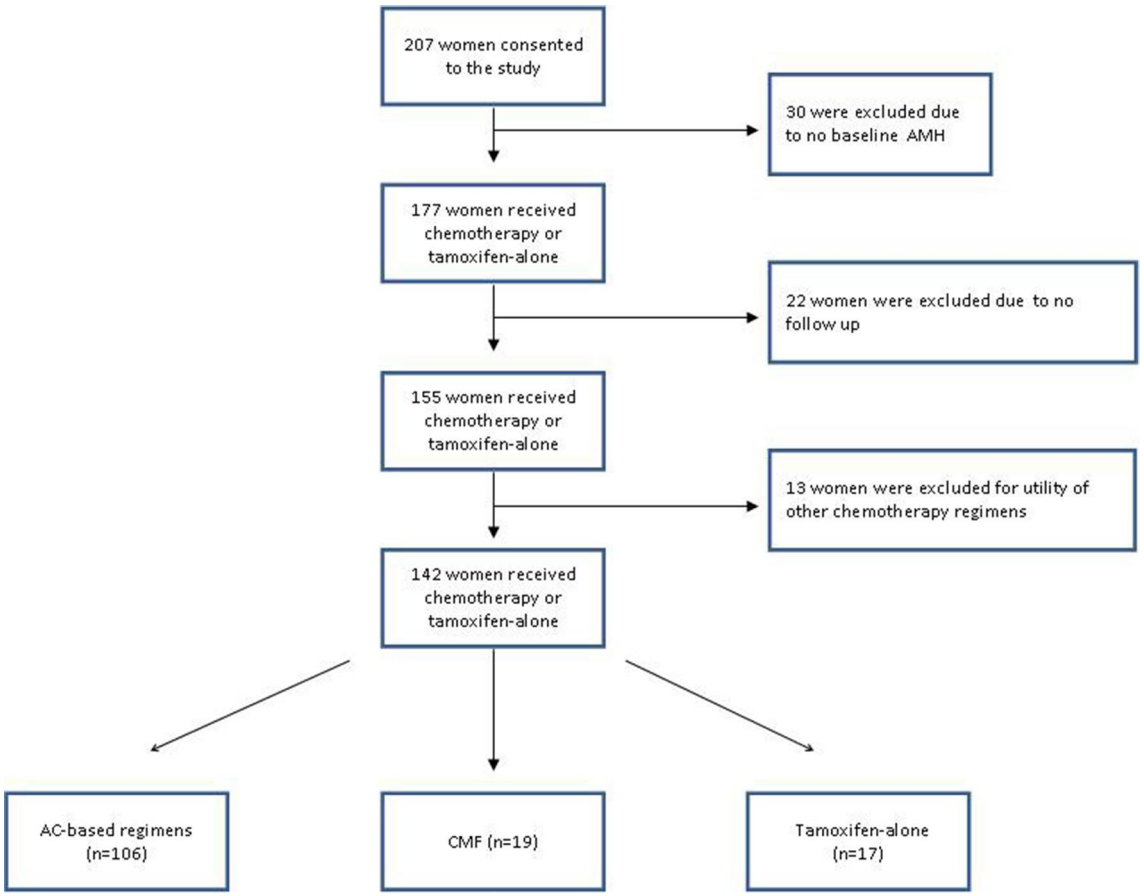

Fig. 1 Study inclusion and exclusions flow chart. *AC* Anthracycline and cyclophosphamide, *CMF* Cyclophosphamide, Methotrexate, 5-Fluorouracil

Comparison of serum AMH levels and recovery among the chemotherapy regimens

Mean ages of women receiving AC-based regimens at recruitment were younger than those of women receiving CMF (36.8 ± 4.5 vs. 40.8 ± 3.4 years; $p = 0.0003$ from t-test), respectively. After chemotherapy, AMH levels were sharply lower at the 12-month time point and this decline was similar among the chemotherapy groups, adjusting for baseline age and BMI (Fig. 2). The mean serum AMH levels were not significantly different between chemotherapy groups at the 12-, 18- and 24-month time points ($p = 0.53$; Fig. 2). In women who received chemotherapy, although the AMH levels continued to recover from 12- up to 24-months post treatment, this recovery was relatively small, and hence, was not clinically meaningful ($p = 0.97$ and 0.04 for 18 and 24 months by signed rank test) (Fig. 3). As an example, while the mean/median AMH level was 0.34/0.09 ng/dl at the 12-month time point, it was 0.40/0.06 ng/dl and 0.42/0.07 ng/dl at 18- and 24-month time points for the AC-based regimens group. These values were 0.11/0.03, 0.12/0.02 and 0.20/0.03 at the 3 time points for CMF group. These mean levels are substantially below the threshold for normal ovarian reserve, which is generally 1.1 ng/mL or higher for the age group. AMH levels remained undetectable in 20% (15/76) vs. 38% (5/13) of women in the AC-based regimens vs. CMF groups at 24 months ($p = 0.16$ from Fisher exact test). AMH recovery rate at 18- and 24-months after the completion of chemotherapy was low in AC-based regimens and CMF groups (mean of 10% and 16% at 18 months and 10% and 7% at 24 months, respectively; median < 4%) (Fig. 2).

The utility of adjuvant tamoxifen treatment did not affect AMH recovery in AC-based regimens. Likewise, HER-2/neu status was also not influential on the outcomes of AC-based regimens. However, we were not able to analyze the effect of adjuvant tamoxifen treatment or HER-2/neu status on women who received CMF due to the small number receiving either.

We previously showed that women with BRCA mutations may be more prone to losing ovarian reserve, due to oocyte DNA repair deficiency [7]. Of the study population, 9.8% carried BRCA mutations, and all were in the AC-based treatment group, with the exception of one. Within this limited

Table 1 Characteristics of 142 study participants that remain after the exclusions

Variables n (%)	AC-based regimens (n = 106)	CMF (n = 19)	Tamoxifen-only (n = 17)
Age	36.8 ± 4.5	40.8 ± 3.4	40.1 ± 3.3
Body mass index	24.9 ± 4.9	22.3 ± 2.9	21.9 ± 3.7
Stage			
(1–2)	77 (72.6)	19 (100)	17 (100)
(3–4)	27 (25.4)	–	–
Unknown	2 (1.8)	–	–
Estrogen receptor status			
Positive	87 (82.1)	19 (100)	17 (100)
Negative	19 (17.9)	–	–
Progesterone receptor status			
Positive	83 (78.3)	18 (94.7)	17 (100)
Negative	23 (21.6)	1 (5.2)	–
HER2 status			
Positive	30 (28.3)	1 (5.2)	–
Negative	76 (71.6)	18 (94.7)	17 (100)
Triple negative	16	–	–
BRCA status			
Positive	13 (12.2)	1 (5.2)	–
Negative	49 (46.2)	10 (52.6)	17 (100)
Untested	44 (41.5)	8 (42.1)	–
Tamoxifen use following chemotherapy			
Yes	80 (75.4)	17 (89.4)	–
No	26 (24.5)	2 (10.5)	

AC Anthracycline-cyclophosphamide, *CMF* Cyclophosphamide, Methotrexate, 5-FU

sample size, the percentages of women with BRCA mutations were 12.3% (13/106) and 5.3% (1/19) in AC-based regimens and CMF groups, respectively.

Impact of tamoxifen-only treatment on serum AMH

Seventeen women who received tamoxifen therapy alone had both a baseline and a follow up serum AMH assessment. The mean age of women who received tamoxifen was 40.1 ± 3.3 at the time of enrollment ($n = 17$). After adjustment for age and BMI at the time of assessment, there was an overall small, but significant decline in serum AMH levels during follow-up, compared to the baseline levels in the tamoxifen group ($p = 0.03$) (Figs. 2, 3). However, this decline is well explained by the expected age-induced decline in AMH levels ($p = 0.79$ for comparing cross-sectional vs. longitudinal effects of age; see Fig. S1 for Observed vs. Expected age trend) [32]. Women using tamoxifen alone had significantly higher AMH levels (measured by slope or time*treatment group interaction) over 24-months compared with women who received chemotherapy ($p < 0.0001$) (Fig. 2).

Discussion

We evaluated the impact of breast cancer chemotherapy and tamoxifen-only treatment on serum AMH levels for a 2-year period. We found that there was a significant decrease in serum AMH regardless of the type of the chemotherapy regimen, but the tamoxifen-only treatment did not affect ovarian reserve. Furthermore, 20% of women in the AC-based regimens and 38% in the CMF group had undetected AMH levels at the end of the two-year follow up period. In contrast, serum AMH levels did not change with tamoxifen treatment during the 24-month follow up beyond natural aging.

One of the key novel findings that our study supplies is the lack of changes in serum AMH levels when tamoxifen was given alone. No other study has investigated serum AMH levels during tamoxifen-only treatment in women who have not received chemotherapy. Likewise, we also did not find any impact of tamoxifen on serum AMH when given subsequent to adjuvant AC-based chemotherapy. While our study is novel with respect to the findings in women receiving tamoxifen-only treatment and its longitudinal design, the impact of tamoxifen treatment on ovarian reserve was evaluated subsequent to chemotherapy in two cross-sectional studies with contrasting results [33, 34]. One study reported lower serum AMH levels in breast cancer survivors who received adjuvant tamoxifen treatment after chemotherapy, but this conclusion was based on a small sample size ($n = 10$) [33]. There was also no adjustment for age as well as the duration of tamoxifen treatment. Another study, in agreement with our findings, reported that post-chemotherapy tamoxifen-users did not have lower ovarian reserve than the tamoxifen-nonusers [34]. In that cross-sectional study, 45 survivors had or were taking tamoxifen, while 63 survivors had not received tamoxifen. After adjusting for age, type and duration of chemotherapy exposure, cancer stage, GnRH agonist use and race, the estimated mean AMH was slightly higher for tamoxifen users.

From the pharmacological point of view, the lack of negative impact on ovarian reserve by tamoxifen is not surprising. Because tamoxifen is not a cytotoxic drug, it is not expected to damage primordial follicle reserve. Tamoxifen is an ovarian stimulant and is sometimes used for ovulation induction in anovulatory patients or for IVF treatments for women with breast cancer [35]. Since the main production source of AMH is mid-size antral follicles and because tamoxifen alters follicle growth dynamics by stimulation, in theory, tamoxifen may spuriously alter and even increase serum AMH levels without affecting primordial follicle reserve. In our study, after accounting for the age-related decline, we did not find a significant change in

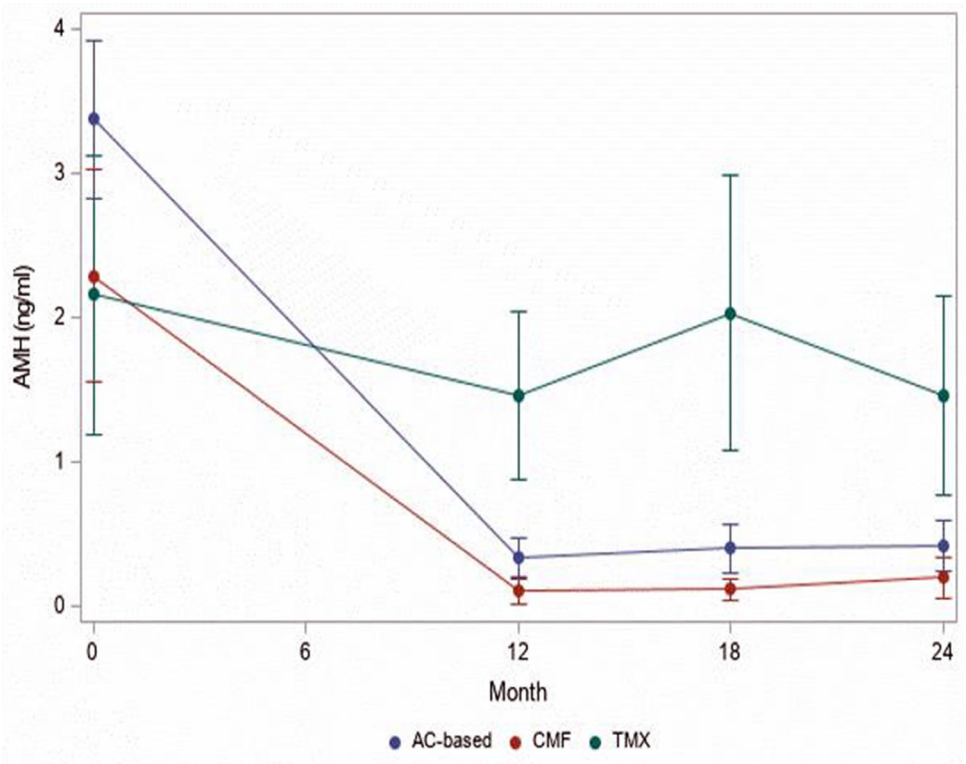

Fig. 2 Longitudinal serum AMH changes 12-, 18- and 24-months after the completion of chemotherapy or tamoxifen-only treatment. After a baseline assessment, 142 women were followed up with 12-, 18- and 24-month serum AMH measurements either after the completion of chemotherapy or during tamoxifen-only treatment. Compared to the baseline, there was a significant decline in serum AMH for those who received chemotherapy at all time points but not for tamoxifen-only treatment group. However, serum AMH levels did not differ at any time point among the women who received AC-based or CMF regimens. Women using tamoxifen-alone showed significantly higher AMH levels at 12-, 18- and 24-months (time-trend or slope captured by time × group interaction) compared with women who received AC-based regimens and CMF (both $p < 0.0001$ vs. tamoxifen). Baseline AMH levels were not statistically different among 3 treatment groups ($p = 0.45$ for df = 2). These comparisons indicate that breast cancer chemotherapy regimens result in significant diminishment of ovarian reserve as early as 12 months after the completion of chemotherapy, but tamoxifen does not seem to alter ovarian reserve, when given alone, beyond natural aging—see Fig. S1 for Observed vs. Expected values of AMH in the tamoxifen group. Vertical bar indicates pointwise 95% confidence interval for crude means (i.e., based on raw data), unadjusted for multiplicity. See Table S1 for summary statistics for each subgroup at different time points. *AC* Anthracycline and cyclophosphamide, *CMF* Cyclophosphamide, Methotrexate, 5-Florouracil, *Tmx* Tamoxifen 20 mg/day

serum AMH with 24 months of follow-up. This suggests that any impact of tamoxifen on growing ovarian follicle populations is compensated during chronic treatment. In conclusion, our novel finding in the tamoxifen-only treatment group, as well as the findings on those who received tamoxifen following chemotherapy, assures us that AMH can be reliably used to monitor ovarian reserve in women who or on long-term tamoxifen treatment.

Our study is one of the few studies that provides longitudinal assessment of ovarian reserve and the only one that assessed chemotherapy-induced ovarian reserve decline at multiple time points during a 24-month post-chemotherapy period. A study by Yu et al. prospectively analyzed changes in Mullerian Inhibiting Substance levels (MIS, former nomenclature for AMH) in 26 women with breast cancer using an earlier less sensitive assay than the one we utilized in our study [36]. While there were multiple time points, the follow up was only for one year from the initiation of chemotherapy and the patients were not stratified based on treatment regimens [36]. The study concluded that breast cancer chemotherapy sharply reduced MIS levels and there was no significant recovery 52 weeks from the initiation or approximately 6-months post-completion of chemotherapy. A later longitudinal study evaluated reproductive function after chemotherapy in 50 premenopausal women with breast cancer, but like the study by Yu et al., patients were only followed for a total of one year including the time spent in treatment [27]. In that study, serum AMH levels were assessed at baseline and every three months for a year during and following chemotherapy. The authors found that there was a rapid decline in serum AMH levels three months after the initiation of chemotherapy without a significant recovery during the one-year follow up. In another longitudinal study, AMH recovery was evaluated by sampling before,

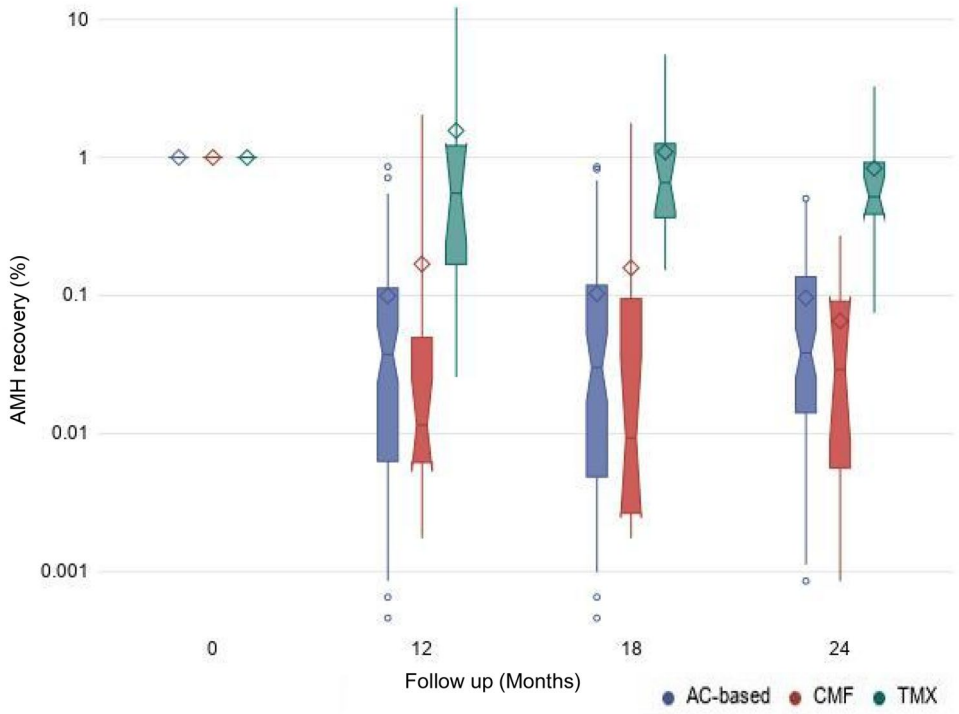

Fig. 3 Whisker Plot of representation of serum AMH recovery rates in women treated with AC-based, CMF and tamoxifen-only treatments during the 12-, 18- and 24-months follow-up. After a baseline assessment, 142 women were followed up for 24-months with serum AMH measurements either after the completion of chemotherapy or during tamoxifen-only treatment. AMH recovery ratio was calculated by dividing follow up AMH values by the baseline value at each time point, where ratio = 1 means full recovery. AMH recovery rate was similar between women who received treatment with AC-based and CMF chemotherapy regimens 12-, 18- and 24-months after the completion of chemotherapy. Tamoxifen treatment alone was associated with higher recovery rates compared with chemotherapy groups at 12-, 18- and 24-months follow-up. See Table S1 for summary statistics for each subgroup at different time points. *AC* Anthracycline and cyclophosphamide, *CMF* Cyclophosphamide, Methotrexate, 5-Floro-uracil, *Tmx* Tamoxifen 20 mg/day

and 4-weeks and 24-months after the initiation of adjuvant chemotherapy but > 50% of those enrolled were on ovarian suppression [37]. Among the 101 women, they found a high rate of ovarian reserve impairment at the 24-month follow up. AMH recovery rates were not measured at any mid time points and women were not stratified based on chemotherapy regimens or adjusted for hormonal suppression. In addition to its larger sample size (127 women had at least a 12-month post-treatment assessment), the novelty of our study is that it provides the longest term follow-up with multiple time points up to 24 months (12-, 18- and 24-month assessment in all 80 women) after the completion of chemotherapy, in addition to the comparison of adjuvant chemotherapy types and the tamoxifen-only treatment.

Our study found that serum AMH levels did not meaningfully recover from 12 to 24 months post-chemotherapy (i.e. increase of < 0.11 ng/ml in mean and nearly 0 in median). Mean serum AMH levels remained well below normal range at all three time points and indicated severely diminished ovarian reserve. These findings suggest that ovarian reserve assessment as early as one year after the completion of chemotherapy should reflect the final ovarian damage incurred. These findings are also consistent with ovarian physiology as it may take 6 months or longer for the surviving primordial follicles to initiate growth and result in developing follicles that can produce AMH again [38].

Previous studies based on amenorrhea rates suggested that the CMF may be more gonadotoxic than AC-based regimens [39]. Our study is the first to compare these protocols with a reliable ovarian reserve marker and did not detect a difference between the AMH recovery patterns of these two chemotherapy regimens. Cyclophosphamide is the most gonadotoxic drug, inducing massive DNA double strand breaks and apoptosis in primordial follicles [4, 40]. Though CMF delivers a larger cumulative dose of cyclophosphamide compared to AC-based regimens, the latter includes doxorubicin, which is also gonadotoxic. We have shown in human ovarian tissue xenograft experiments that doxorubicin also induces DNA double strand breaks and triggers apoptotic death of primordial follicles similar to cyclophosphamide [4, 40]. The inclusion of two gonadotoxic drugs in the AC-based regimen may explain its gonadotoxic equivalency to CMF, despite the utility of larger cyclophosphamide doses in the latter. However, we remain reserved with this conclusion

as the sample size was smaller for the CMF group and the percentage of women with undetectable AMH were higher with the latter. Moreover, all but one of the women with BRCA mutations were in the AC-based group, which we previously showed to associate with lower AMH recovery [7].

Despite its novelty and numerous strengths, our study also had some limitations. We did not know the smoking status of approximately 30% of participants and smoking can result in lower serum AMH levels [41]. Though we could not adjust our analysis for smoking status due to the missing information, the incidence of smoking was only 20% in the remaining 70% of the population studied. Given this low incidence and the fact that the study longitudinally compared AMH changes in reference to subjects' own baseline AMH, smoking status is unlikely to affect our results. We also did not screen the study population for polycystic ovarian syndrome, a disease which could be associated with spuriously elevated AMH levels. However, since we excluded women with irregular periods or amenorrhea from participation and since AMH recovery is permuted relative to each subject's baseline measurements, it is unlikely that PCOS-screening could have altered our results. Finally, CMF and tamoxifen groups had relatively low sample sizes, and the power of our conclusions is relatively limited regarding these two treatment groups.

In summation, our study is the first to assess ovarian reserve changes with serum AMH in a prospective longitudinal fashion, with multiple time points up to 24-month post-completion of chemotherapy, and in comparison with tamoxifen-only treatments. It shows that the common chemotherapy regimens used for breast cancer treatment are highly detrimental to future reproductive potential, but the tamoxifen-only treatments do not seem to affect serum AMH assessment. However, larger studies may be needed to determine the precise differences of gonadotoxicity between the specific chemotherapy regimens and the impact of tamoxifen treatments on AMH-measurement accuracy. In the meantime, our study provides novel information to counsel young women with breast cancer for fertility preservation before they receive adjuvant chemotherapy and tamoxifen-only treatments.

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Author contributions Conception of the idea: KO; Design: KO, MD, SG, SP; Study execution: SG, MD, KO, GB, VT, ET**;** Provision of study materials: KO, MD, SG, TC**;** Manuscript writing: KO, VT, SG, GB, HB; Statistical Analysis: VT, HB**;** Final approval: All authors.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval 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.

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