International Journal of Research in Medical Sciences
Özkan O et al. Int J Res Med Sci. 2026 Mar;14(3):791-797
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20260590

Original Research Article

Ovarian reserve and markers after chemotherapy in young women with early-stage breast cancer

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Received: 30 November 2025
Revised: 05 January 2026
Accepted: 12 February 2026

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ABSTRACT

Background: The aim of the study was to compare ovarian reserve and its markers between young women who received chemotherapy for early-stage breast cancer and healthy controls.
Methods: This cross-sectional study was conducted at Gülhane Military Medical Academy (GATA, currently Ankara Gülhane Training and Research Hospital), Ankara, Türkiye, between December 2010 and October 2011. Ovarian reserve was evaluated in 34 women with early-stage breast cancer who resumed regular menstruation after chemotherapy and compared with 34 age- and gravida-matched healthy controls. Main outcome measures included antral follicle count (AFC), total ovarian volume, anti-Müllerian hormone (AMH), FSH, and estradiol (E2) levels on days 2 or 3 of the menstrual cycle.
Results: The median AFC was 5 in the cancer group and 7 in the controls. AMH, AFC, ovarian volume, and FSH levels significantly differed between groups, favoring the controls. A significant difference in E2 levels was also noted. AFC and AMH were strongly correlated (r=0.84 and r=0.74, respectively). Multivariate logistic regression identified AFC as the strongest predictor of AMH.
Conclusions: Chemotherapy for early-stage breast cancer is associated with reduced ovarian reserve in young women despite the continuation of menstruation.

Keywords: Breast cancer, Ovarian reserve, Chemotherapy, Fertility

INTRODUCTION

Global estimates from 2020 indicate that there were 2.3 million new cases of breast cancer, representing approximately 12% of all newly diagnosed cancer cases worldwide, and resulting in 685,000 deaths. Advances in screening techniques have led to an increase in the detection of breast cancer, while mortality rates, particularly among younger populations, have seen a decline in recent years.[1]

Adjuvant chemotherapy, routinely administered to women with early-stage breast cancer, improves both disease-free survival and overall survival. However, breast cancer chemotherapy is associated with ovarian toxicity, which may compromise a woman's future fertility. While some women experience complete follicular depletion and develop permanent ovarian failure during chemotherapy, others may become temporarily amenorrheic, with menstrual cycles resuming months or even years after treatment cessation. However, the mere return of menstrual cycles is not a reliable indicator of normal ovarian function.[2]

Accurately identifying patients at risk of infertility or those who respond poorly to chemotherapy can help physicians

tailor counseling and assist patients in understanding their chances of achieving pregnancy.

Reproductive potential is closely related to both the quantity and quality of ovarian primordial follicles, both of which decline with age. Well-known risk factors for chemotherapy-induced ovarian failure include age, the specific chemotherapy regimen, subsequent use of endocrine therapy, and genetic predispositions.[3] Several tests are available to assess ovarian reserve during and after chemotherapy, including follicular phase serum levels of estradiol (E2), follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH), inhibin B (InB), antral follicle count (AFC), and ovarian volume.[3] However, the extent to which these measurements reliably assess fertility in cancer patients remains unclear.

The aim of the study was to assess ovarian reserve measurements in women who underwent chemotherapy for early-stage breast cancer and maintained regular menstrual cycles following treatment.

METHODS

Study design and setting

This cross-sectional study was conducted at Gülhane Military Medical Academy (currently Ankara Gülhane Training and Research Hospital), Ankara, Türkiye. The aim was to evaluate ovarian reserve in women who had undergone chemotherapy for early-stage breast cancer and resumed menstruation, compared to age- and gravida-matched healthy controls.

Eligible breast cancer patients were identified through a retrospective review of medical records of women under 40 years old who had received chemotherapy for early-stage (Stage I-II) or locally advanced (Stage IIIa) breast cancer between 2000 and 2011. Among 138 identified patients, 94 were contacted by phone, and 42 were excluded due to persistent amenorrhea. The remaining 52 women were invited for evaluation, and 34 meeting the inclusion criteria were included in the study group. The sample size was determined based on the number of eligible patients available during the study period. The control group consisted of 34 healthy women without any known gynecological pathology, selected from individuals who visited Ankara Gülhane Training and Research Hospital for routine gynecological check-ups.

Inclusion and exclusion criteria

Inclusion criteria

Participants were eligible for inclusion if they met the following criteria as patients with age below 40 years, a documented history of chemotherapy administered for Stage I to IIIa breast cancer, and resumption of regular menstrual cycles (defined as at least three consecutive cycles) following the completion of chemotherapy.

Exclusion criteria

Patients were excluded if they met any of the following conditions currently receiving adjuvant hormonal therapy (e.g., gonadotropin-releasing hormone analogues or tamoxifen); less than one year elapsed since the end of chemotherapy; diagnosis of advanced-stage or recurrent breast cancer; history of adnexal surgery or the presence of adnexal pathology; and failure to achieve at least three consecutive post-treatment menstrual cycles.

Breast cancer staging was conducted in accordance with the TNM classification system established by the American Joint Committee on Cancer (AJCC). Relevant demographic and clinical data, including chemotherapy regimens and the timing of administration, were retrospectively obtained from electronic medical records.

Hormonal and ultrasound assessment

Blood samples were collected on the 2nd, 3rd, or 4th day of the menstrual cycle to assess serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) using the electrochemiluminescence method. Anti-Müllerian hormone (AMH) levels were measured using enzyme-linked immunosorbent assay (ELISA).

Transvaginal ultrasound (TVS) was performed during the early follicular phase to assess ovarian volume and count antral follicles (2–10 mm) in both ovaries. Total antral follicle count (AFC) and ovarian volume were calculated. All ultrasound examinations were conducted by the same experienced obstetrician to minimize inter-observer variability.

Statistical analysis

Data were analyzed using SPSS for Windows, Version 15.0. Normality of distribution was assessed using the Kolmogorov-Smirnov test.

For normally distributed data, comparisons between groups were made using Student's t-test; for non-normally distributed data, the Mann-Whitney U test was used. Spearman's correlation test was used to assess associations between AFC and serum hormone levels. One-way ANOVA and Kruskal-Wallis tests were applied for multiple group comparisons depending on data distribution.

A p value<0.05 was considered statistically significant.

Ethical considerations

The study was approved by the Gülhane Military Medical Academy (GATA) Local Ethics Committee, Ankara, Türkiye (Approval No: 1491-157-11). Written informed consent was obtained from all participants prior to their inclusion in the study.

RESULTS

Participant characteristics

The study, conducted between December 2010 and October 2011, included 34 reproductive-age women who had undergone chemotherapy for early-stage breast cancer and 34 healthy controls. The ovarian reserves of the cases were assessed both biochemically and ultrasonographically.

Table 1 presents the demographic characteristics of the study group and controls. The average age of the study group was 35±2.8 years, while the average age of the control group was 36±3.1 years (p=0.74).

The average age of the study group prior to starting treatment was 30.2±2.75 years. All women were married, with 2 women in the study group and 1 woman in the control group being nulliparous. All participants had received radiotherapy.

During chemotherapy, menstruation ceased in 91% (n=31) of the cases but resumed after the completion of treatment. In patients who did not receive hormone therapy (n=33, 38%), menstruation resumed within the first year. In patients who were estrogen receptor-positive and received hormone therapy (GnRHa for 3 years and tamoxifen for 5 years) (n=21, 62%), regular menstruation resumed immediately after the completion of therapy. Among women in the study group, 79% (n=27) used contraceptive methods. No fertility preservation procedures were performed on women in the study group (data not shown in the table).

Breast cancer staging

Among the women in the study group, 20% (n=7) were classified as stage I, 47% (n=16) as stage IIa, 18% (n=6) as stage IIb, and 15% (n=5) as stage IIIa. The majority of the cases were early-stage breast cancer (85%, n=29), while 15% (n=5) were classified as locally advanced breast cancer.

Ovarian reserve post-chemotherapy

Table 2 displays the ovarian reserve parameters of women who resumed menstruation after chemotherapy. The study findings indicate that while antral follicle count (AFC) and ovarian volume decreased, and FSH and LH levels increased with advancing stage, these changes were not statistically significant.

The study also categorized the time since treatment as follows: 15% had less than 3 years since diagnosis, 44% had between 3 and 5 years, and 41% had more than 6 years since diagnosis. Despite the reduction in ovarian reserve over time, no significant relationship was found between the duration since treatment and ovarian reserve parameters.

Ovarian reserve by chemotherapy regimen

Table 3 presents the ovarian reserve parameters according to the chemotherapy regimens administered in the study group. No significant differences were found in ovarian reserve parameters based on the chemotherapy regimen received. Furthermore, no significant differences were observed when comparing ovarian reserve parameters between patients who received hormone therapy (n=21) and those who did not (n=13) (data not shown in the table).

Ovarian reserve in age groups

When groups were analyzed based on age, distinguishing between those below and above 35 years, ovarian reserve parameters were found to be better in those under 35 years of age. Additionally, ovarian reserve parameters in the control group were significantly better compared to the study group. Table 4 illustrates these findings. Furthermore, when evaluating ovarian reserve based on the age at which treatment was initiated, it was observed that ovarian reserve significantly decreased in patients aged 30 years and older compared to those under 30 years (data not shown in the table).

Ovarian reserve parameters comparison: study verses control group

Univariate logistic analyses demonstrated statistically significant differences in AMH, AFC, FSH levels, and ovarian volume between the study group and the control group. Specifically the average antral follicle count (AFC) was significantly higher in the control group compared to the study group (7.03 versus 5.06, p=0.005); the average ovarian volume was markedly lower in the study group than in the controls (7.2 versus 9.7, p<0.001); the controls exhibited significantly higher average AMH levels compared to the study group (0.88 versus 0.35, p=0.021); the median FSH level was significantly lower in the controls compared to the study group (7.3 versus 11.3, p<0.001); and no statistically significant difference was observed in estradiol (E2) levels between the two groups (Table 5).

Correlation of ovarian reserve parameters

In the analysis of correlations among ovarian reserve parameters, a strong positive correlation was observed between ovarian volume and AFC with AMH (r=0.84 and r=0.74, p<0.001).

Conversely, a weaker negative correlation was found between FSH and the other parameters (r=-0.34, p=0.004). The correlations of estradiol (E2) and LH with other parameters were found to be weak (Table 6).

Predictors of ovarian reserve parameters

The multiple logistic regression model identified AFC as the best predictor of AMH levels (Table 6).

Table 1: Characteristics of the study and control groups.

Characters	Mean±SD		P value
Mean age (years)	35.88±2.84	36.12 ±3.13	0.746
BMI	25.24±2.34	25.52±1.52	0.565
Gravida	1±0.69	1±0.78	0.495
Parity	1±0.61	1±0.61	0.836
Smoking	0±0.35	0±0.41	1.00
Age at menarche (years)	13±1.13	13±0.92	0.573
Mean age (years)	35.88±2.84	36.12 ±3.13	0.746

Note: Comparisons between groups were made using Student's t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. A p value<0.05 was considered statistically significant.

Table 2: Ovarian reserve parameters by breast cancer stage in the study group.

Evre	Satı (N)	Ovarian reserve parameters (mean)						
		Age (year)	AFC (n)	AMH (ng/ml)	Ovarian volume (cm^3)	FSH (Miu/ml)	e2 (pg/ml)	LH (mIU/ml)
Evre I	7	33.00	4.29	0.08	6.36	9.67	92.00	5.59
Evre IIa	16	30.25	4.81	0.43	7.32	10.36	80.69	6.79
Evre IIb	6	28.5	5.83	0.35	7.65	11.37	69.00	6.94
Evre IIIa	5	28.80	5.06	0.34	7.22	10.34	80.21	6.36
P		0.007	0.047	0.07	0.62	0.84	0.74	0.363
Control group	34	36.12	7.03	0.88	9.74	7.30	77.52	5.02

Note: Comparisons between groups were made using Student's t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. A p value<0.05 was considered statistically significant.

Table 3: Ovarian reserve parameters by chemotherapy regimens in the study group.

Chemotherapy Regimens	N	Ovarian reserve parameters [mean (median)]						
		Age	AMH (ng/ml)	AFC	Ovarian Volume (cm^3)	FSH (mIU/ml)	E2 (pg/ml)	LH (mIU/ml)
CA	5	32.6	0.20 (0.18)	4.40 (4.32)	6.44 (6.08)	10.69 (10.08)	108 (101)	6.42 (6.08)
CA-T	3	29.7	0.43 (0.40)	600 (580)	7.55 (7.09)	10151 (9918)	53 (49)	7.00 (6.99)
CAF-T	3	30.5	0.22 (0.40)	5.33 (5.00)	6.78 (6.28)	11.49 (11.00)	73 (72)	5.00 (4.78)
CAF	19	29.3	0.40 (0.37)	5.32 (5.18)	6.95 (6.65)	10.31 (9.98)	74 (70)	6.70(6.60)
TAC	4	30.3	0.22 (0.19)	3.75 (3.10)	7.22 (7.01)	10.58 (9.99)	100 (97)	5.20 (5.05)
P		0.348	0.283	0.654	0.829	0.840	0.315	0.440

Note: Comparisons between chemotherapy regimens were made using one-way ANOVA for normally distributed data and Kruskal-Wallis test for non-normally distributed data. A p value<0.05 was considered statistically significant. (doksorubisin ve siklofosfamid (CA). doksorubisin. siklofosfamid. 5-fluorourasil (CAF). doksorubisin. siklofosfamid-taxan (CA-T). doksorubisin. siklofosfamid. 5-fluorourasil-taxan (CAF-T). doksorubisin. siklofosfamid taxan (TAC).

Table 4: Comparison of control and study groups by age below and above 35 years.

Parameters	Control group			Study group	
		Mean (median)	P value	Mean (median)	P value
Ovarian volume (cm^3)	≤35	11.67 (10.76)	<0.001	8.43 (8.12)	0.001
	>35	8.21 (8.08)		6.26 (6.08)	
AFC	≤35	9.93 (9.76)	<0.001	6.60 (6.09)	<0.001
	>35	4.74 (4.12)		3.84 (3.90)	
FSH (m/Ulml)	≤35	6.93 (6.89)	0.453	9.17 (9.99)	0.041
	>35	7.59 (7.80)		11.27 (11.08)	

Continued.

Parameters	Control group			Study group	
		Mean (median)	P value	Mean (median)	P value
AMH (nglml)	≤35	1.68 (1.60)	<0.001	0.66 (0.76)	<0.001
	>35	0.24 (0.16)		0.09 (0.07)	
E2 (pg/ml)	≤35	64.73 (63.71)	0.041	58.27 (56.05)	<0.001
	>35	87.63 (87.70)		97.53 (97.99)	

Note: Comparisons between groups were made using the Mann-Whitney U test for non-normally distributed data. A p value<0.05 was considered statistically significant.

Table 5: Differences in ovarian reserve parameters between the study group and the control group.

Olgu		Mean	Median	Minimum	Median	P value
Total antral follicle count						
Control group	34	7.03±3.26	7.00	1.00	14.00	0.05
Study group	34	5.06±2.26	5.00	0	9.00	
Total ovarian volume (cm³)						
Control group	34	9.74±2.38	9.87	5.14	14.85	<0.001
Study group	34	7.22±1.96	6.74	4.44	11.84	
AMH (ng/ml)						
Control group	34	0.88±1.06	0.34	0.045	3.96	0.021
Study group	34	0.35±0.52	0.14	0.034	2.49	
FSH (mlu/ml)						
Control group	34	7.30±2.46	7.16	2.33	13.92	<0.001
Study group	34	11.34±3.11	9.90	5.45	20.20	
LH (mlu/ml)						
Control group	34	5.02±2.58	4.33	1.95	14.10	0.1016
Study group	34	6.39±1.94	6.34	1.86	11.23	
E2 (pg/ml)						
Control group	34	77.52±62.79	52.50	21	295	0.15
Study group	34	80.20±38.31	68.50	24	185	

Note: Comparisons between groups were made using Student's t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. A p value<0.05 was considered statistically significant.

Table 6: Correlations among ovarian reserve parameters.

Parameter	Pearson Correlation	P value
AFC- over volume	0.84	<0.001
AFC-FSH	-0.49	<0.001
AFC- LH	-0.18	0.136
AFC- EZ	-0.292	0.016
AFC-AMH	0.743	<0.001
Ovarin volume- FSH	-0.482	<0.001
Ovarin volume- LH	-0.184	0.133
Ovarin volume- EZ	-0.209	0.088
Ovarin volume- AMH	0.707	<0.001
FSH- LH	0.461	<0.001
FSH- EZ	0.10	0.33
FSH- AMH	-0.341	0.004
LH- EZ	0.124	0.312
LH- AMH	-0.159	0.196

Note: Correlations between ovarian reserve parameters were assessed using Pearson's correlation coefficient. A p-value<0.05 was considered statistically significant.

DISCUSSION

In line with our aim to evaluate ovarian reserve after chemotherapy in young breast cancer survivors, our findings demonstrated significantly reduced ovarian reserve markers, particularly AMH and AFC, compared to age-matched healthy controls. Data on reproductive outcomes for women who have survived breast cancer are limited. However, recent population-based cohort studies have shown that young women who have completed breast

cancer treatment are 46% more likely to be diagnosed with infertility compared to their cancer-free counterparts.[4] Our findings are consistent with those reported by Baxter et al., who observed a reduction in birth rates among women with breast cancer (Hazard Ratio-HR 0.74; 95% CI 0.61, 0.91).[5]

Although effective options for fertility preservation are available, recent studies have shown that the referral rates for fertility preservation among young women diagnosed with breast cancer remain low.[6,7] In our study, none of the patients in the study group underwent fertility-preserving interventions. Despite the reduction in fertility capacity due to breast cancer treatment, unnecessary fertility preservation procedures for women who will remain fertile after chemotherapy and hormone therapy can lead to waste of time and resources, as well as unwarranted exposure of cancer cells to exogenous sex hormones.

AHM levels are one of the best predictors of ovarian reserve in the general population. Lower AMH levels are associated with reduced success in in vitro fertilization.[8] Several studies have investigated AMH as a marker of ovarian dysfunction following breast cancer treatment. A recent study analyzing data from 84 patients found that a pre-chemotherapy AMH level >3 ng/mL (area under the curve=0.69, 95% CI: 0.54-0.84) was the best predictor of post-chemotherapy AMH ≥0.7 (sensitivity=79%, specificity=60%.[9] Another study observed ovarian function recovery in 62 participants (57%) after an average follow-up of 163 days post-chemotherapy. Adjusted analyses showed that AMH levels >0.7 ng/ml (HR 2.9, 95% CI 1.5-5.6) and FSH levels ≤10 IU/l (HR 4.7, 95% CI 1.3–16.8) were associated with a shorter time to ovarian recovery, while Inhibin B levels were not significantly related.[10] Additionally, as emphasized in these studies, amenorrhea is not a reliable measure of ovarian function, and regular menstrual cycles are not an appropriate indicator of ovarian reserve.[8-10]

Despite the interest in serum markers such as AMH, FSH, Inhibin B (InB), and estradiol (E2), antral follicle count (AFC) is considered to be the most accurate reflection of ovarian reserve.[11-13] A prospective study examining the effects of various chemotherapy regimens on AMH levels, AFC, and ovarian volume (OV) found that while AMH levels and AFC significantly decreased one year after chemotherapy ($p<0.0001$), OV did not change significantly ($p=0.507$). Additionally, patients who developed amenorrhea had lower AMH and AFC levels compared to those who did not develop amenorrhea. There was no significant difference in chemotherapy regimens (including or excluding taxanes) regarding the development of amenorrhea. These findings are consistent with our results.[12] Furthermore, our study identified that ovarian volume (OV) is a valuable measurement, correlating well with AFC and other ovarian reserve parameters. A retrospective analysis involving 107 patients conducted in 2020 indicated that amenorrhea induced by chemotherapy in breast cancer patients was significantly associated with age during treatment, age at menarche, and use of tamoxifen. The resumption of menstruation was linked to younger age (<40 years), later menarche (≥13 years), or not using tamoxifen.[14] In our study, age during treatment was also evaluated as one of the most influential factors on ovarian function.

In this study, the evaluation of ovarian reserve based on the chemotherapy regimens did not reveal significant differences, likely due to the small number of patients across different regimens. A study assessing ovarian reserve in relation to chemotherapy protocols found that the CMF regimen was more gonadotoxic compared to the CAF/CEF or CA protocols.[15] However, CMF therapy is no longer commonly used today. Taxanes, on the other hand, are widely used in clinical cancer treatment, but specific reproductive toxicological information remains debated.[16] Initial clinical studies suggested that the addition of taxanes to anthracycline-based chemotherapy increased the likelihood of amenorrhea.[17] Recent clinical studies have demonstrated that taxane monotherapy has a strong ovarian toxic effect by evaluating amenorrhea and serum steroid hormone levels in premenopausal patients.[18,19]

This study has several limitations. First, the relatively small sample size may have limited the statistical power to detect differences across chemotherapy regimens. Second, the retrospective design introduces the risk of selection bias and incomplete data collection. Third, the absence of longitudinal follow-up restricts our ability to evaluate long-term ovarian function recovery.

In summary, this study provides further evidence that ovarian reserve decreases after adjuvant chemotherapy for breast cancer, consistent with previous research. Young women with early-stage breast cancer face significant gynecological impacts, including effects on fertility, menstrual regularity, risks of ovarian and endometrial cancer, early menopause, and osteoporosis. Therefore, it is essential to tailor adjuvant therapies for these women based on tumor characteristics, coexisting medical conditions, financial factors, and personal acceptance of recommended treatments. This approach optimizes treatment strategies to better meet the individual needs and circumstances of patients.[20]

CONCLUSION

Our study shows that ovarian reserve declines more rapidly in young women who have undergone chemotherapy for early-stage breast cancer than in healthy individuals. These findings highlight the importance of fertility counseling and careful consideration of the potential side effects of hormonal and chemotherapeutic treatments in this population.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Özkan O, Akduman AT, Duru NK. Ovarian reserve and markers after chemotherapy in young women with early-stage breast cancer. Int J Res Med Sci 2026;14:791-7.