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의학박사 학위논문

Effect of Timing of Gonadotropin-releasing Hormone Agonist Administration on Ovarian Reserve in Patients with Premenopausal Breast Cancer

폐경 전 유방암 환자에서 항암 치료 중 생식샘자극호르몬분비호르몬작용제의 투여 시점에 따른 난소 보호 효과

2020년 8월

서울대학교 대학원

의학과 산부인과 전공

신 재 준

Abstract

Effect of Timing of Gonadotropin-releasing Hormone Agonist Administration on Ovarian Reserve in Patients with Premenopausal Breast Cancer

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Introduction: Gonadotropin-releasing hormone agonist (GnRHa) is used to suppress the ovaries for fertility preservation during cancer chemotherapy. However, there is an initial paradoxical 'flare-up' phase that increases the release of gonadotropins and increase the ovarian activity. It is unknown whether or not GnRHa have ovarian protective effect if administered during this

period. The aim of this study was to investigate the effect of the interval between the start of GnRHa and the start of chemotherapy on ovarian protection in patients with breast cancer.

Methods: This study used the data from a prospective observational cohort study that included 136 patients with breast cancer below 40 years who received GnRHa during chemotherapy for fertility preservation. Plasma anti-Müllerian hormone (AMH) levels were measured before chemotherapy (baseline) and after chemotherapy. Subjects were divided into three groups according to the interval between the start of GnRHa and the start of chemotherapy for analysis: 1–6 days, 7–13 days, and ≥14 days. The percentage change of the post-chemotherapy AMH value to the baseline AMH (pcAMH) at each time point were compared among the three groups. Ranked analysis of covariance was used for statistical analysis, adjusted for age, body mass index (BMI), and the existence of polycystic ovaries (PCO). In addition, live

birth after chemotherapy was also assessed among the three groups. Factors associated with recovery of ovarian function (AMH ≥ 1 ng/mL) at 12 months was also evaluated.

Results: The median age of the patients was 32 years. There was no difference in the baseline AMH levels among the three groups (mean ± standard error, 5.0 ± 0.4 ng/ml [1–6 days], 5.3 ± 0.7 ng/ml [7–13 days], and 8.1 ± 1.3 ng/ml [≥14 days], p = 0.250). The pcAMH at 3, 6, 12, 24, and 36 months were not significantly different among the three groups (p values were 0.332, 0.732, 0.830, 0.148, and 0.393, respectively). Among 69 married women, 21 delivered (30.4%). There was no difference in the proportion of delivered women among the three groups (p = 0.680), and there was also no significant difference in the live birth among the three groups using Kaplan-Meier plot and the log rank test (p = 0.999). In multivariate analysis, young age (p = 0.024), low BMI (p = 0.013), and the existence of PCO (p = 0.015)

were predictors for AMH ≥ 1 ng/mL at 12 months.

Conclusion: There was no difference in the ovarian protective effect according to the difference in the timing of administration of GnRHa.

Keywords: Anti-Müllerian hormone, Breast cancer, Chemotherapy, Fertility preservation, Gonadotropin-releasing hormone

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I. Introduction

Preserving fertility has become an important issue in young women who are breast cancer survivors. Breast cancer is the most commonly diagnosed cancer in young women aged <40 years. The 5-year survival rate in this age group has been reported to be 85% (Fidler *et al.*, 2017, Zolton *et al.*, 2018). Young breast cancer survivors may then want to conceive, but it can be too late for them at that point.

Women are born with a finite number of oocytes, and the number of oocytes at a time point is called the germ cell pool or the ovarian reserve. From menarche to menopause, the ovarian reserve progressively decreases over the years without possibility for renewal. The depletion of the ovarian reserve is accelerated by chemotherapeutic agents, resulting in transient amenorrhea, infertility, or permanent loss of ovarian function (menopause).

The probability of ovarian damage is greater in young patients diagnosed with breast cancer since they are more likely to receive cytotoxic chemotherapy due to the aggressiveness of the disease at that age than older patients. The risk for ovarian damage is higher with old age, poor ovarian reserve before chemotherapy, and is dependent on the

type, duration, and total dosage of chemotherapeutic agents (Brydoy *et al.*, 2007).

The ovarian reserve cannot be measured directly, and various parameters have been used in previous studies to assess it (Clemons and Simmons, 2007). Menstrual status (presence or absence of regular monthly menstruation) is the most widely used method, although it is not the most ideal marker of ovarian reserve. There is no unified definition for amenorrhea, and the duration of amenorrhea used in the definition of ovarian failure markedly varies from 3 to 12 months among studies (Zavos and Valachis, 2016). Furthermore, amenorrhea does not always represent permanent ovarian failure because patients may experience transient amenorrhea for a long time after chemotherapy. On the other hand, restoration of regular menstruation does not always represent sufficient ovarian reserve. Patients with regular, albeit short menstrual periods may have diminished ovarian reserve (Klein *et al.*, 1996, Xue *et al.*, 2019). The measurement of follicle-stimulating hormone is limited due to the fact that it has to be tested only in the early follicular phase of the menstrual cycle and can be easily affected by hormonal medications administered to the patient. A more accurate measurement of ovarian function after chemotherapy was needed to both assess ovarian function and to aid selection

of better endocrine therapy after chemotherapy.

Measurement of serum anti-Müllerian hormone (AMH), which is produced by the granulosa cells of small growing follicles, has proven to be the most accurate indicator of the antral follicle pool, indirectly reflecting the remaining ovarian reserve (Freour *et al.*, 2017, Perdrix *et al.*, 2017). It shows a constant value regardless of which phase of the menstrual cycle the sample was taken. In addition to reflecting post-chemotherapy damage, AMH is capable of predicting ongoing ovarian activity after chemotherapy (van Rooij *et al.*, 2005, Anderson *et al.*, 2012, Sandow *et al.*, 1978, Dewailly *et al.*, 2014). Moreover, it is less affected by the administration of gonadotropin-releasing hormone agonist (GnRHa) than other indicators, which makes it an ideal marker of ovarian reserve in cases where GnRHa is used. However, AMH is also known to be affected by the presence of the polycystic ovarian (PCO) morphology on ultrasound (Laven *et al.*, 2004, Pigny *et al.*, 2006, Pellatt *et al.*, 2007, Homburg *et al.*, 2013). Therefore, when using AMH as a surrogate marker for ovarian reserve, the presence or absence of PCO morphology must be considered during analysis.

Cryopreservation of oocytes and embryos following oocyte retrieval is the only established method for fertility preservation in breast cancer; however, it cannot be applied to

all patients and there is need for other medical therapies (Practice Committee of American Society for Reproductive, 2013). Although the efficacy of GnRHa for fertility preservation is controversial, recent research has supported its efficacy and safety (Lambertini *et al.*, 2019).

The use of GnRHa induces an initial release of gonadotropins that induces ovulation and increase the ovarian estradiol production (i.e., "flare-up phase") followed by desensitization of gonadotropin-releasing hormone receptors in the pituitary gland, which then blocks the secretion of gonadotropins (Sandow *et al.*, 1978). The flare-up phase usually lasts for several days; however, it is unknown whether GnRHa still has an ovarian protective effect if chemotherapy is initiated during this flare-up phase.

The flare-up phase usually lasts for several days; however, it is unknown whether GnRHa still has an ovarian protective effect if chemotherapy is initiated during this flare-up phase.

Therefore, in this study, we investigated the efficacy of the GnRHa when first administered at different time points before the start of chemotherapy.

II. Materials & methods

II-1. Study population and participants

This was a prospective observational study. Women with cancer who were scheduled to receive chemotherapy and desired counseling for future fertility were referred to the fertility clinic at Seoul National University Hospital, Seoul, Korea. As a routine procedure, before counseling at the clinic, all subjects underwent pelvic ultrasound examination and plasma AMH levels were evaluated to identify women with decreased ovarian reserve. Initially, oocyte/embryo/ovary tissue cryopreservation was recommended for eligible subjects. Subsequently, ovarian suppression with monthly administration of long-acting GnRHa (Goserelin 3.6 mg subcutaneous injection, Zoladex®, AstraZeneca, London, UK) during chemotherapy was offered to the appropriate candidates. Plasma AMH levels were evaluated at regular intervals after the completion of chemotherapy, if needed, along with a description of patients' menstrual status. Since October 2009, the fertility preservation cohort included various patients with cancer who decided to receive long-acting GnRHa for fertility preservation. The cohort did not receive any intervention, and the treatments, laboratory exams, and follow-ups were performed as per the physician's judgement.

From this large cohort, operable premenopausal breast cancer patients who were ≤ 40 years old and diagnosed between October 2009 and February 2016 were eligible for this study. Premenopausal status before chemotherapy was confirmed by spontaneous menstruation, visible follicles on pelvic ultrasound examination, and premenopausal hormone status.

To ensure full coverage of the monthly administration of GnRHa treatment over chemotherapy, only patients who received GnRHa at least 1 day before the initiation of chemotherapy and at least once after the last dose of chemotherapy were included. Patients who did not have relevant medical records (n = 21), who did not receive chemotherapy as planned (n = 3), who received sequential GnRHa to prevent breast cancer recurrence (n = 5), and who underwent ovarian stimulation after initiation of GnRHa (n = 1) were excluded.

Various chemotherapeutic regimens were used: the 2 most common regimens were 5-fluorouracil (5-FU, 500 mg/m^2) plus doxorubicin (50 mg/m^2) and cyclophosphamide (500–600 mg/m^2) every 3 weeks for 6 cycles and doxorubicin (60 mg/m^2) plus cyclophosphamide (600 mg/m^2) every 3 weeks for 4 cycles, followed by 4 cycles of paclitaxel (175 mg/m^2) or docetaxel (75 mg/m^2). The regimens used were classified into

anthracycline-based, anthracycline-taxane-based, taxane-based, or others including CMF (6 cycles of cyclophosphamide [50 mg per oral thrice a day for 14 days] plus methotrexate [40 mg/m^2 on days 1 and 8] plus 5-FU [600 mg/m^2 on days 1 and 8] every 4 weeks).

Plasma AMH was measured using a commercially available kit (Gen II ELISA, Beckman Coulter, Brea, CA, USA). The assay has a measurement range of 0.08–22.50 ng/mL. The intra- and inter-assay coefficient of variation were 5.4% and 5.6%, respectively. AMH values ≤ 0.08 ng/mL were imputed to one-half the threshold value (0.04 ng/mL) in the analyses.

The number of follicles in each ovary and the ovarian volume recorded in the initial pelvic ultrasound examination were used to identify polycystic ovaries (PCOs). The PCO morphology was defined as the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 cm^3) on transvaginal or transrectal ultrasound examination (Balen *et al.*, 2003).

The primary endpoint was to compare ovarian function among different timings of GnRHa administration. The main outcome measure was the percentage change in post-chemotherapy plasma AMH (pcAMH) level, which was calculated using the following equation: .

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

Patients were divided into 3 groups according to the interval between the initiation of GnRHa and chemotherapy ($T_{interval}$, 1–6, 7–13, or ≥14 days).

Along with the assessment of ovarian function, the most definite way of demonstrating a woman's fertility is her ability to conceive. Pregnancy after chemotherapy, if described in the medical records, were used for analysis.

In addition, recovery of ovarian function, defined as AMH ≥ 1.0 ng/mL, was evaluated based on previous reports (Nelson *et al.*, 2007, Lee *et al.*, 2018).

II-2. Statistical analysis

Continuous variables were expressed as mean ± standard error (SE), and the median values were shown when necessary. Baseline continuous variables were compared using the Kruskal-Wallis test. Qualitative variables were expressed as frequencies and percentages and compared using the χ^2 test. Potential confounders of pcAMH were evaluated using correlation analysis. Ranked analysis of covariance (ANCOVA) was used to compare the pcAMH values at different time points in each of the 3 groups while adjusting for the covariates

identified in the correlation analyses. A multivariate analysis was performed to identify factors that were predictors of the recovery of ovarian function (AMH ≥ 1.0 ng/mL). Statistical significance was defined as differences with $p < 0.05$. All analyses were performed using SPSS version 23 (IBM Corporation, Armonk, USA).

II-3. Ethics statement

The Institutional Review Board (IRB) of Seoul National University Hospital (Seoul, Korea) approved the prospective cohort, and informed consent was obtained from each patient (study registered as H-0802-043-234).

III. Results

A total of 136 patients were included in the present analysis (Figure 1). The baseline characteristics of the subjects are summarized in Table 1. The median age was 32 years, and most subjects were nullipara (82.4%). The median follow-up duration of plasma AMH was 24 months. GnRHa was administered for an average of 158.6 days. Patients with hormone receptors positive tumors were common in the present cohort (79.3%), and most of the hormone receptor positive patients received adjuvant tamoxifen (95.3%).

Before comparison of three $T_{interval}$ groups, a correlation analysis was performed to identify possible confounding factors. In the correlation analysis, age (Pearson coefficient -0.414, $p < 0.001$), body mass index (BMI, Pearson coefficient -0.251, $p = 0.015$), and PCO on ultrasound (Spearman coefficient 0.358, $p = 0.001$) were independently correlated with the AMH level (Table 2). Therefore, the pcAMH values shown in Table 3 were compared after adjustment for age, BMI, and PCO. Of note, the chemotherapy regimens and the use of tamoxifen did not demonstrate a significant correlation with AMH level.

Baseline characteristics were similar among the 3 groups, except for the polycystic ovary (PCO) morphology on

ultrasound (p = 0.042), where the PCO morphology was significantly more frequent in the 14-days-or-more group than in the 1-6-days group in the post-hoc analysis (p = 0.013). The overall prevalence of PCO morphology was 32.4% (44/136 patients)(Table 3).

The AMH and pcAMH were nearly undetectable at 3 months and increased slowly thereafter, as shown in Figure 2(a). The pcAMH levels at each time point following chemotherapy was not significantly different among the 3 $T_{interval}$ groups (Table 3 and Figure 2).

In order to investigate the difference in pcAMH according to PCO, we divided the subjects into two groups according to the presence/absence of PCO, and the results are shown in Table 4 and Figure 3. Regardless of the presence/absence of PCO, there was no difference in pcAMH among the three $T_{interval}$ groups a any given time point. In Figure 3(a), overall, the pcAMH showed a moderate recovery over time. The data showed 63.03% recovery in the 7-13 days group, and 63.73% recovery in the 1-6 days group at 36 months. However, there was only one subject in the 1-6 days group, and there were 7 subjects in the 7-13 days group and the results should be interpreted with caution.

Further analysis to investigate whether there is a

difference in the recovery of pcAMH after chemotherapy (Table 5 and Figure 4). The difference in pcAMH between women with PCO and without PCO was statistically not significant from 3 to 24 months, but the difference was statistically significant at 36 months (p = 0.047).

The reproductive outcomes of the patients are shown in Table 6. Based on the available medical records, among the 69 women who were married either before or after chemotherapy, 21 women (30.4%) delivered after chemotherapy. Figure 5. shows the number of patients delivered during the follow up duration. Their number were few, but many of them delivered between 4-7 years after chemotherapy.

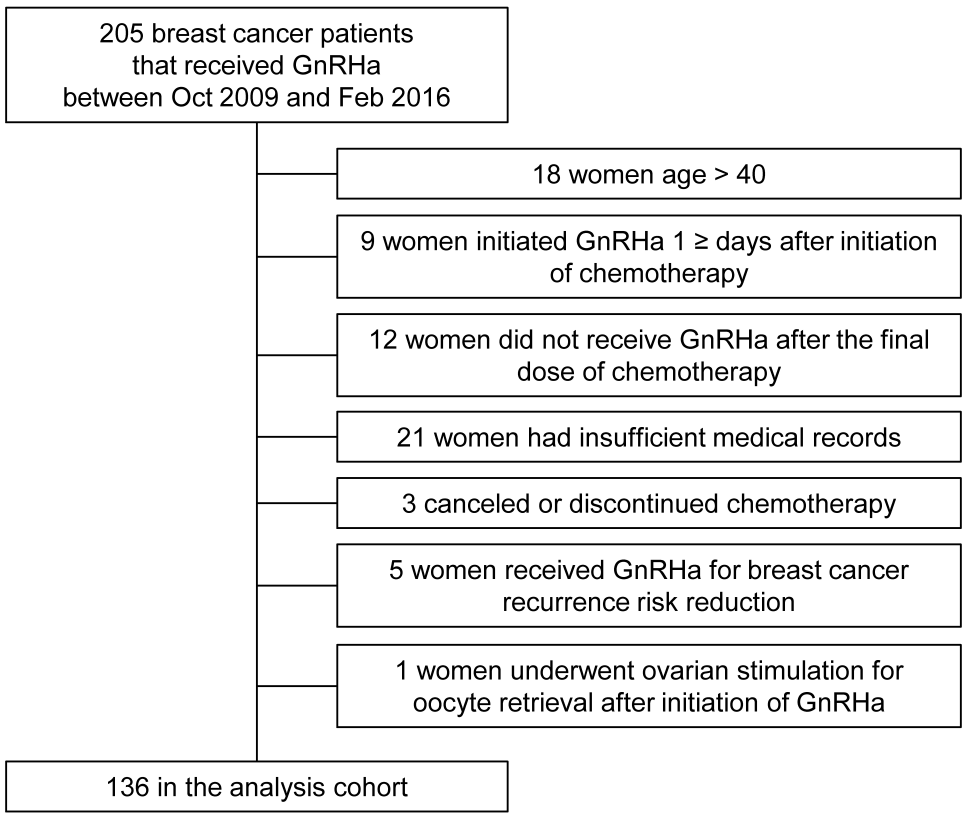
There was no difference in the proportion of women who delivered after chemotherapy among the three $T_{interval}$ groups (p = 0.680, Table 7.) Comparison among the three groups using the Kaplan-Meier plot and Log-Rank test also did not demonstrate a significant difference among the three groups (p = 0.999, Figure 6.)

The assessment of predictive factors for delivery after chemotherapy using the available data is shown in Table 8. The only factor that showed a positive correlation was the age at the diagnosis of breast cancer, and the younger the age at diagnosis, the higher the possibility to conceive and deliver

after chemotherapy (p = 0.009).

The factors associated with AMH ≥ 1 ng/mL at 12 months are summarized in Table 9. The proportion of patients with AMH ≥ 1 ng/mL at 12 months was 44.2%. Young age (adjusted odds ratio [OR] 0.87; 95% confidence interval [CI] 0.77–0.98]), low BMI (adjusted OR 0.81; 95% CI 0.68–0.96), and presence of PCO (adjusted OR 3.86; 95% CI 1.30–11.44) were associated with AMH ≥ 1 ng/mL. However, tamoxifen use and chemotherapy regimen did not have a significant association with AMH ≥ 1 ng/mL.

Figure 1. Flow diagram of subject selection



GnRHa = gonadotropin-releasing hormone agonist.

Table 1. Patient Characteristics

Parameter	Value
Age at diagnosis (years)	32 (19-39)
Age at menarche (years)	13 (10-16)
BMI (kg/m^2)	21.5 ± 0.3
Baseline antral follicle count	16.2 ± 1.0
Baseline AMH (ng/mL)	5.6 ± 0.4
Duration of GnRHa administration (days)	158.6 ± 3.4
$T_{interval}$ (days)	9.4 ± 0.7
Parity	
0	112 (82.4)
1	22 (16.2)
2	2 (1.5)
Married	52 (39.0)
PCO morphology on ultrasound	44 (32.8)
Histopathological type	
IDC	118 (86.8)
Mucinous carcinoma	5 (3.7)
Metaplastic	3 (2.2)
Mixed IDC and invasive lobular carcinoma	3 (2.2)
Mixed IDC and mucinous carcinoma	2 (1.5)
Invasive lobular carcinoma	2 (1.5)
Others	2 (1.5)
Type of surgery	
Breast-conserving surgery	91 (66.9)
Mastectomy	45 (33.1)

Initial clinical stage	
IA	38 (27.9)
IB	0 (0)
IIA	44 (32.4)
IIB	22 (16.2)
IIIA	23 (16.9)
IIIB	1 (0.7)
IIIC	5 (3.7)
IV	3 (2.2)
Chemotherapy protocol	
Adjuvant	105 (77.2)
Neoadjuvant and adjuvant	31 (22.8)
Chemotherapy regimen	
Anthracycline-based	66 (48.5)
Anthracycline-taxane-based	67 (49.3)
Others	3 (2.2)
Hormone receptor status	
ER positive and/or PR positive	107 (79.3)
ER negative and PR negative	28 (20.7)
HER2 receptor positive	17 (15.3)
Tamoxifen	102 (75.0)
Trastuzumab	17 (15.2)

Data are shown as mean ± standard error, median (range), or number (%).

PCO = polycystic ovary; BMI = body mass index; IDC = invasive ductal carcinoma; GnRHa = gonadotropin-releasing

hormone agonist; AMH = anti-Müllerian hormone; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; $T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy.

Table 2. Identification of covariates for anti-Müllerian hormone at 12 months

	Correlation coefficient	*p*-value
Age	-0.414*	< 0.001
BMI	-0.251*	0.015
PCO morphology on ultrasound	0.358†	0.001
Tamoxifen	0.029†	0.781
Chemotherapy regimen‡	-0.044†	0.675

BMI = body mass index; PCO = polycystic ovary.

*Pearson correlation coefficient.

†Spearman correlation coefficient.

‡Two group analysis: anthracycline-based chemotherapy versus anthracycline and taxane-based chemotherapy.

Table 3. Comparison of characteristics, AMH and pcAMH* according to $T_{interval}$

	$T_{interval}$			
	1-6 days (n = 50)	7-13 days (n = 64)	14 days or more (n = 22)	*p*-value
Age (year)	33 (22-40)	33 (20-40)	32 (22-40)	1.000†
BMI (kg/m^2)	22.2 ± 0.5	20.9 ± 0.3	21.6 ± 0.7	0.314†
Antral follicle count	14.0 ± 1.4	16.9 ± 1.5	18.7 ± 2.7	0.297†
Subjects with PCO morphology on ultrasound	12 (24.5)	20 (31.3)	12 (54.5)	0.042‡
Chemotherapy regimen				0.554‡
Anthracycline	22 (44.0)	35 (54.7)	9 (40.9)	
Anthracycline and taxane	27 (54.0)	27 (42.2)	13 (59.1)	
Others	1 (2.0)	2 (3.1)	0 (0)	
Tamoxifen	39 (78.0)	46 (71.9)	17 (77.3)	0.728‡

Time till resumption of menstruation after chemotherapy (days)	206.5 ± 17.9	266.8 ± 22.6	264.6 ± 52.1	0.191†
Pre-chemotherapy AMH (ng/mL)	5.0 ± 0.4	5.3 ± 0.7	8.1 ± 1.3	0.250§
pcAMH* (%)				
3 months (n = 127)	4.4 ± 1.3	5.5 ± 1.1	3.4 ± 1.2	0.332§
6 months (n = 115)	16.9 ± 6.6	13.1 ± 3.2	10.0 ± 3.3	0.732§
12 months (n = 95)	22.7 ± 4.5	29.7 ± 6.2	32.8 ± 10.1	0.830§
24 months (n = 73)	26.1 ± 5.3	28.0 ± 5.2	33.7 ± 7.5	0.148§
36 months (n = 35)	34.7 ± 15.6	30.9 ± 8.0	24.9 ± 8.2	0.393§

Data are shown as mean ± standard error, median (range), or number (%).

BMI = body mass index; PCO = polycystic ovary; AMH = anti-Müllerian hormone; pcAMH = percentage change in post-chemotherapy anti-Müllerian hormone; $T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy.

*pcAMH was calculated by the equation:

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

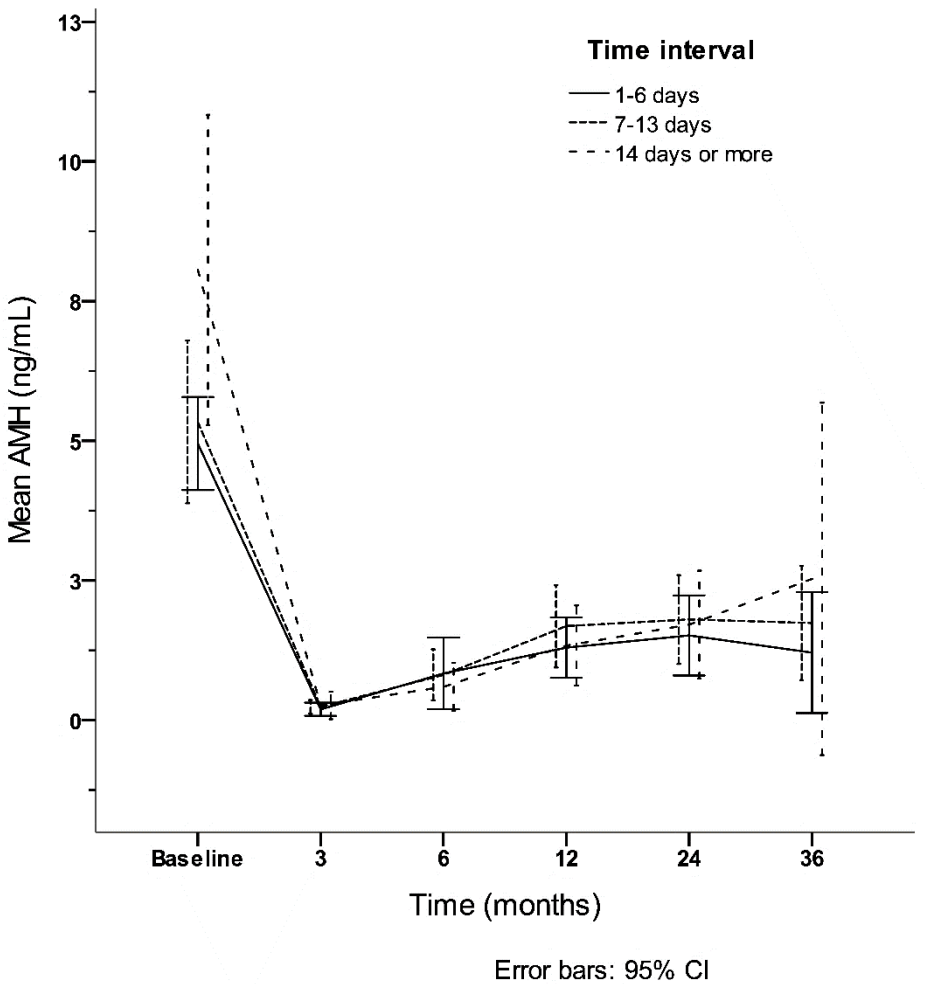
†Kruskal–Wallis test.

‡Chi-square test.

§ Ranked ANCOVA test, adjusted for age, BMI, and PCO.

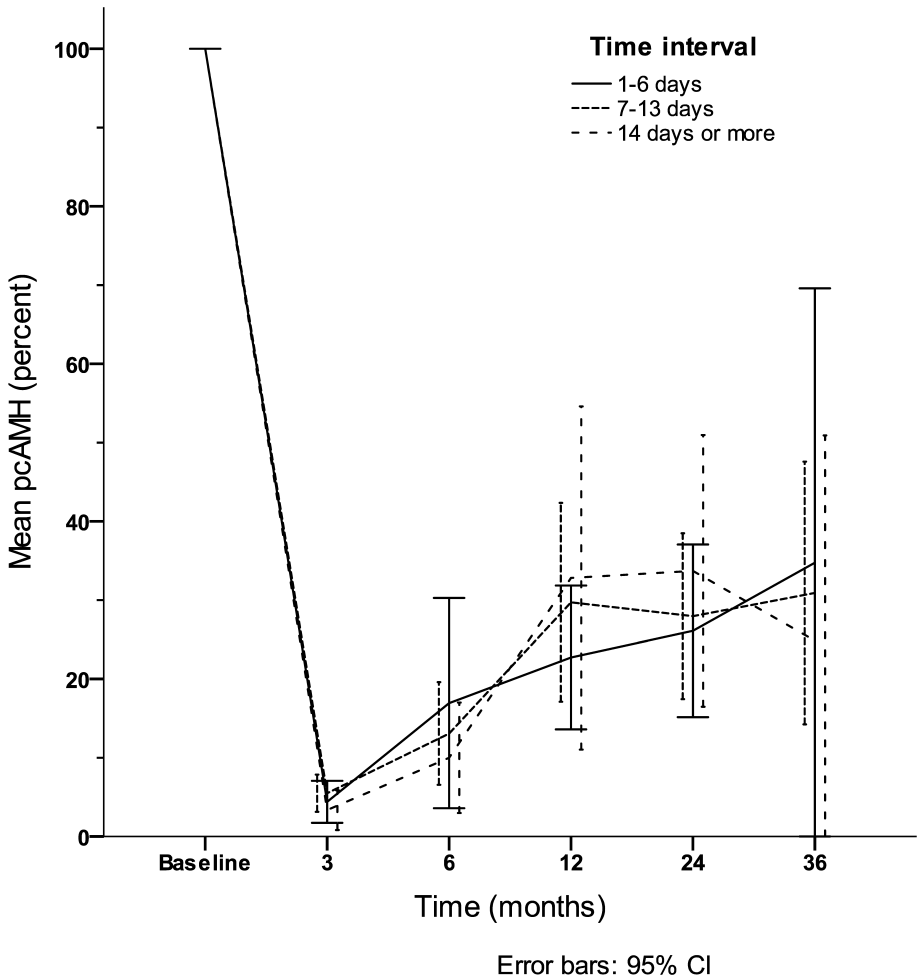
Figure 2. Comparison of changes in variables before and after chemotherapy among the three $T_{interval}$ groups. Error bars represent 95% confidence interval.

(a) AMH



(b) pcAMH. The pcAMH was calculated by the equation.

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$



AMH = anti-Müllerian hormone; pcAMH = percentage change in post-chemotherapy anti-Müllerian hormone; $T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy

Table 4. Comparison of pcAMH* according to $T_{interval}$, in women with PCO and without PCO

pcAMH* (%)	$T_{interval}$ 1-6 days	7-13 days	14 days or more	*p*-value
With PCO				
3 months	2.51 ± 0.82 (n=11)	7.08 ± 2.58 (n=19)	3.78 ± 2.15 (n=9)	0.440†
6 months	7.85 ± 2.86 (n=11)	25.92 ± 8.62 (n=16)	5.87 ± 2.79 (n=11)	0.267†
12 months	20.76 ± 3.98 (n=11)	49.07 ± 13.82 (n=13)	26.37 ± 14.84 (n=6)	0.907†
24 months	20.68 ± 6.88 (n=8)	49.29 ± 11.85 (n=13)	38.73 ± 13.39 (n=3)	0.118†
36 months	63.73 (n=1)	63.03 ± 16.55‡ (n=7)	41.21 (n=1)	0.890†
Without PCO				
3 months	5.06 ± 1.73 (n=37)	4.78 ± 1.26 (n=42)	2.86 ± 0.94 (n=8)	0.494†
6 months	20.01 ± 8.95 (n=33)	7.02 ± 1.91 (n=34)	15.01 ± 6.44 (n=9)	0.282†
12 months	23.69 ± 6.28 (n=28)	20.74 ± 5.97 (n=28)	37.65 ± 14.31 (n=8)	0.574†
24 months	27.14 ± 7.32 (n=18)	16.40 ± 2.96 (n=24)	31.19 ± 9.72 (n=6)	0.373†
36 months	29.65 ± 18.84 (n=9)	13.62 ± 3.08 (n=13)	19.41 ± 8.63 (n=3)	0.540†

Data are shown as mean ± standard error.

PCO = polycystic ovary; AMH = anti-Müllerian hormone; pcAMH = percentage change in post-chemotherapy anti-Müllerian hormone; $T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy.

*pcAMH was calculated by the equation:

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

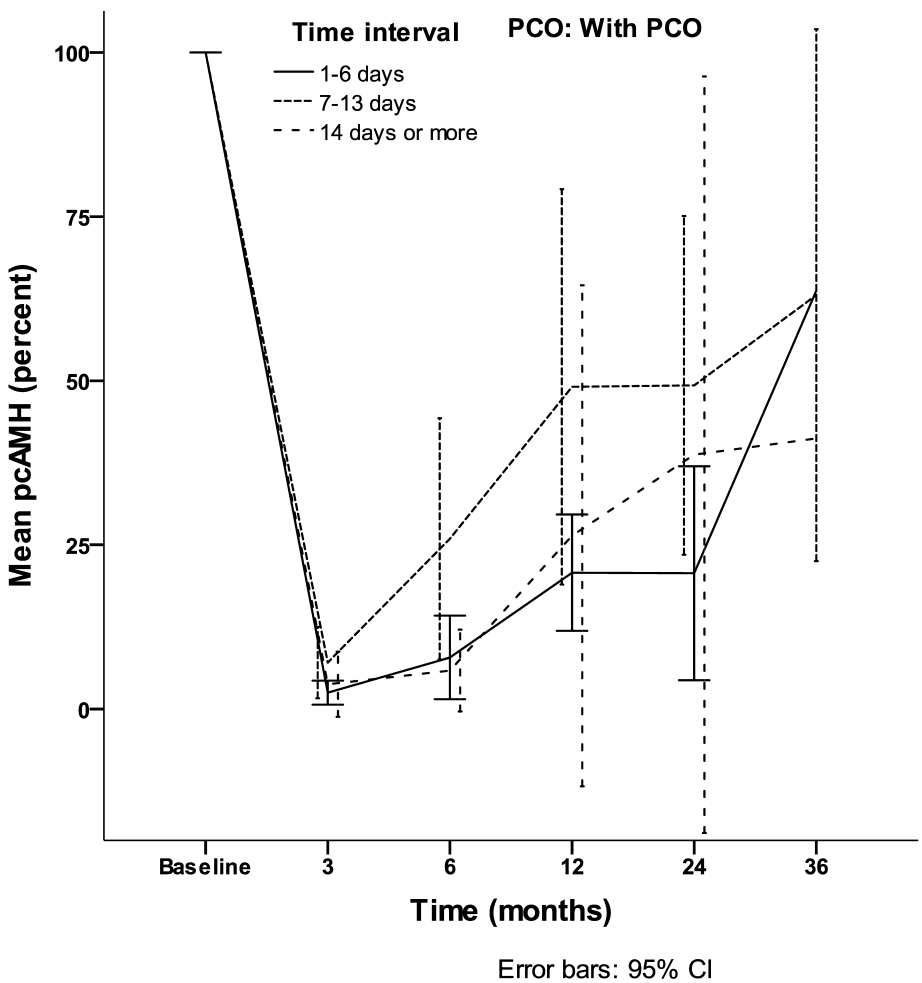
†Ranked ANCOVA test, adjusted for age, BMI (body mass index).

‡Actual data: 19.23, 23.84, 40.23, 50.44, 60.05, 122.05, 125.35

Figure 3. Comparison of changes in pcAMH before and after chemotherapy among the three $T_{interval}$ groups, (a) with PCO and (b) without PCO. Error bars represent 95% confidence interval. The pcAMH was calculated by the equation:

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

(a) with PCO



(b) without PCO

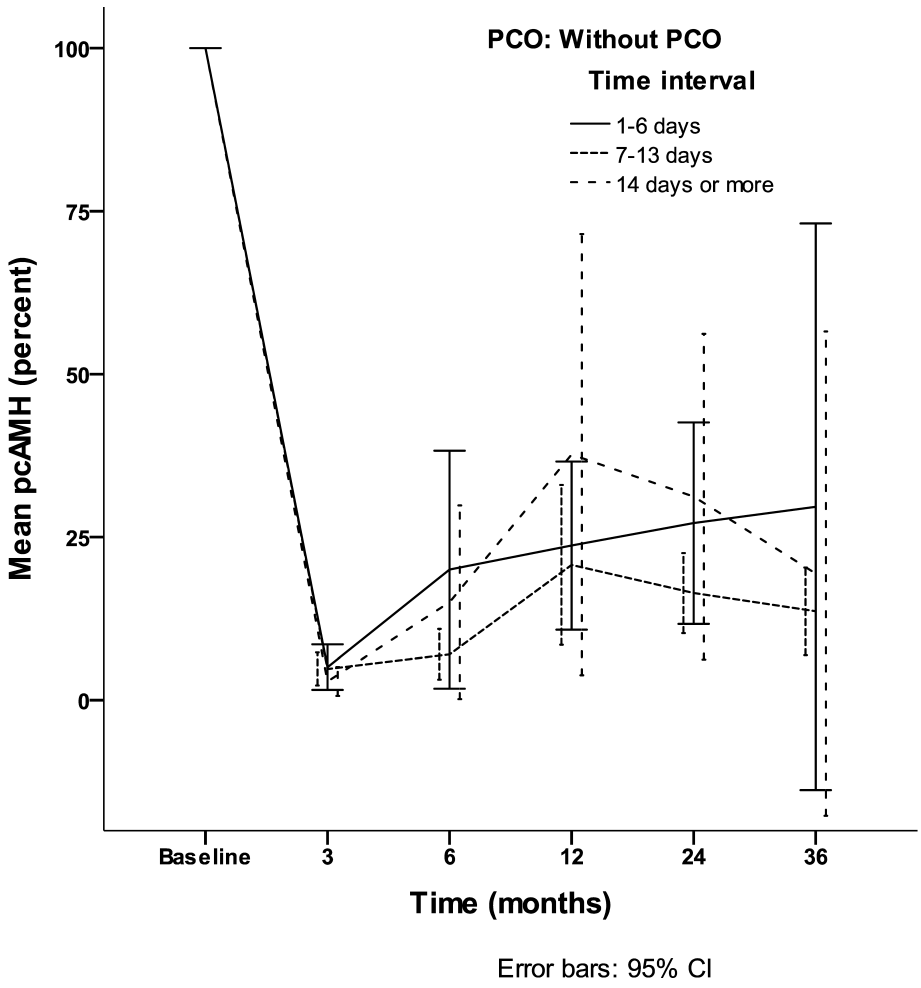


Table 5. Comparison pcAMH* according to PCO.

pcAMH* (%)	With PCO	Without PCO	*p*-value
3 months	5.03 ± 1.39 (n = 39)	4.72 ± 0.95 (n = 87)	0.810†
6 months	14.89 ± 4.04 (n = 38)	13.61 ± 4.07 (n = 76)	0.725†
12 months	34.15 ± 7.06 (n = 30)	24.14 ± 4.17 (n = 64)	0.794†
24 months	38.43 ± 7.33 (n = 24)	22.28 ± 3.38 (n = 48)	0.368†
36 months	60.68 ± 12.88 (n = 9)‡	20.08 ± 6.94 (n = 25)	0.047†

Data are shown as mean ± standard error.

PCO = polycystic ovary; AMH = anti-Müllerian hormone; pcAMH = percentage change in post-chemotherapy anti-Müllerian hormone;

*pcAMH was calculated by the equation:

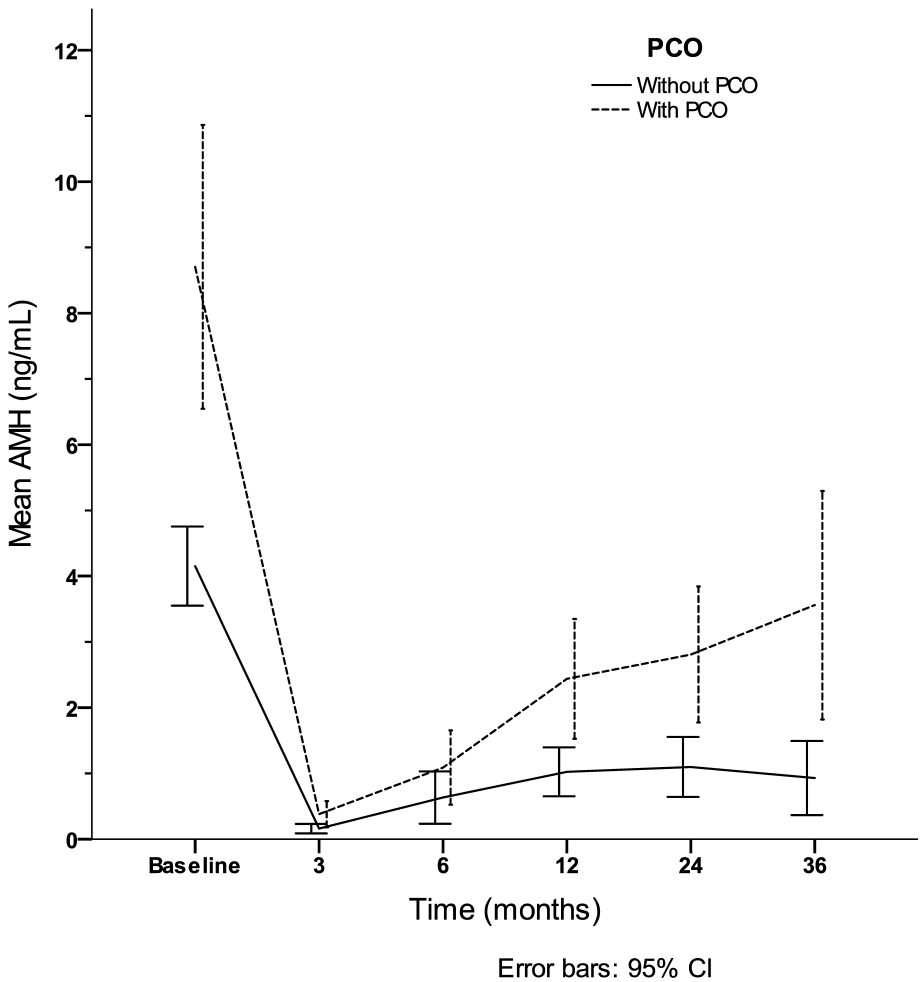
$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

†Ranked ANCOVA test, adjusted for age, BMI (body mass index)

‡Actual data: 19.23, 23.84, 40.23, 41.21, 50.44, 60.05, 63.73, 122.05, 125.35

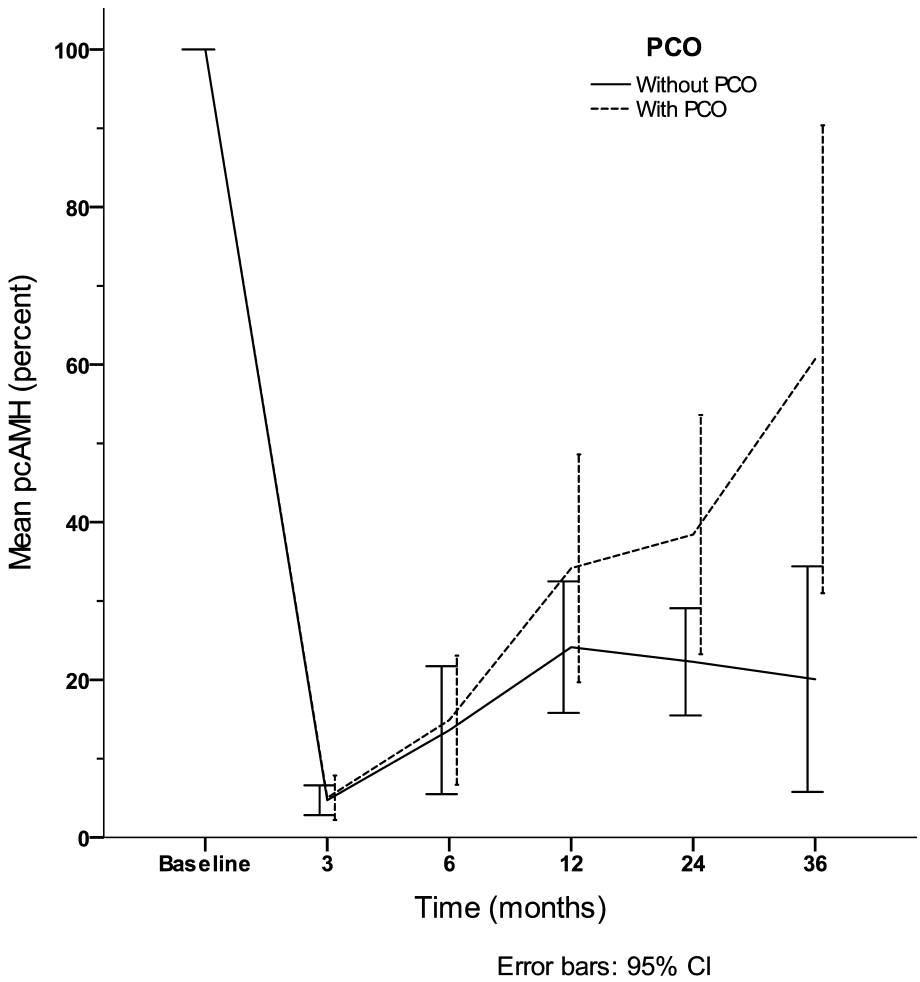
Figure 4. Comparison of changes in variables before and after chemotherapy between women with PCO and without PCO. Error bars represent 95% confidence interval.

(a) AMH



(b) pcAMH. The pcAMH was calculated by the equation:

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$



PCO = polycystic ovary; AMH = anti-Müllerian hormone; pcAMH = percentage change in post-chemotherapy anti-Müllerian hormone

Table 6. Reproductive outcome

Status	N	(%)
Married before chemotherapy	48	(35.3)
Live birth after chemotherapy	13	(9.6)
No live birth after chemotherapy	35	(25.7)
Unmarried before chemotherapy	88	(64.7)
Married after chemotherapy	21	(15.4)
Live birth after chemotherapy	8	(5.9)
No live birth after chemotherapy	13	(9.6)
Remained unmarried after chemotherapy	59	(43.4)
Marital status unknown after chemotherapy	8	(5.9)

Figure 5. Delivery and follow up period after chemotherapy

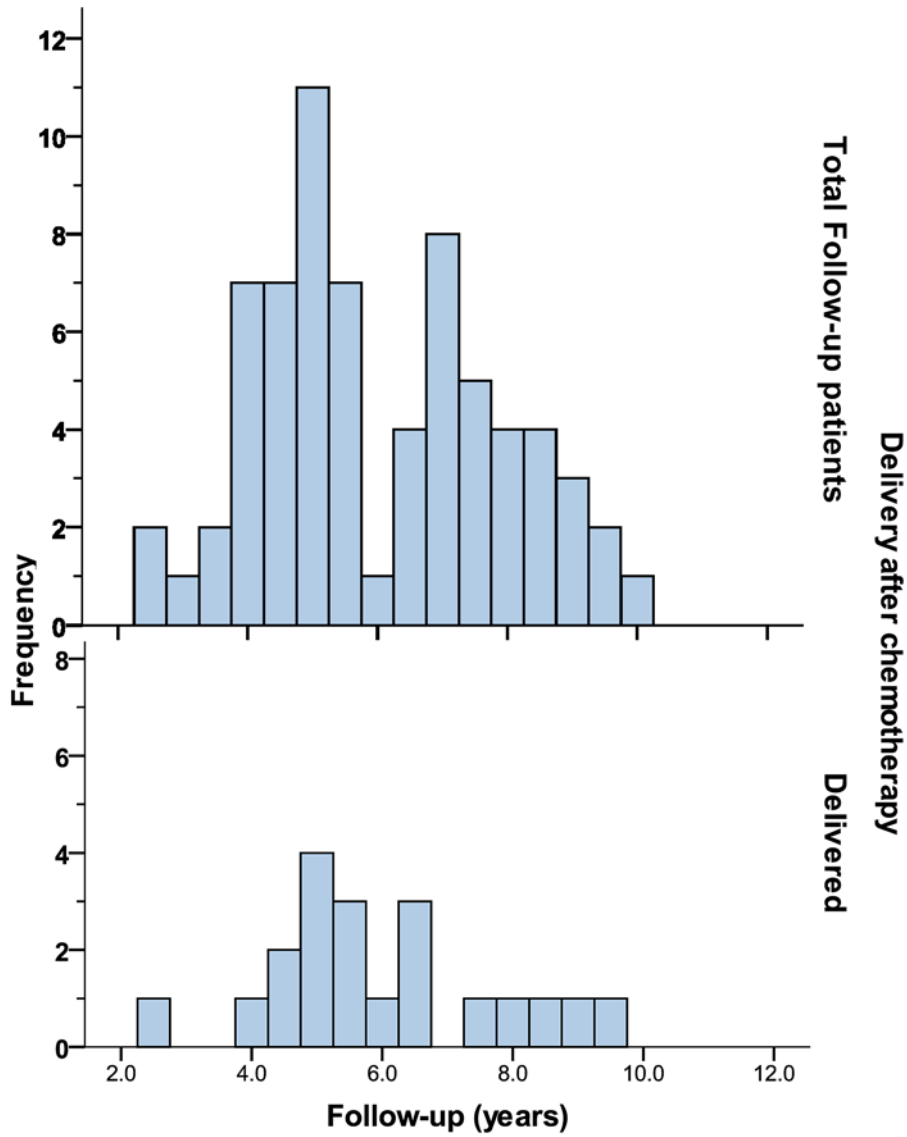


Table 7. Comparison of live birth in the three $T_{interval}$ groups

	$T_{interval}$			
	1-6 days	7-13 days	14 days or more	*p*-value
Live birth after chemotherapy	7 (30.4)	11 (34.4)	3 (21.4)	0.680*
No live birth after chemotherapy	16 (69.6)	21 (65.6)	11 (78.6)	

$T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy

*Chi-square test.

Figure 6. Kaplan-Meier plot of cumulative live birth after chemotherapy (Log-Rank test *p*-value: 0.999)

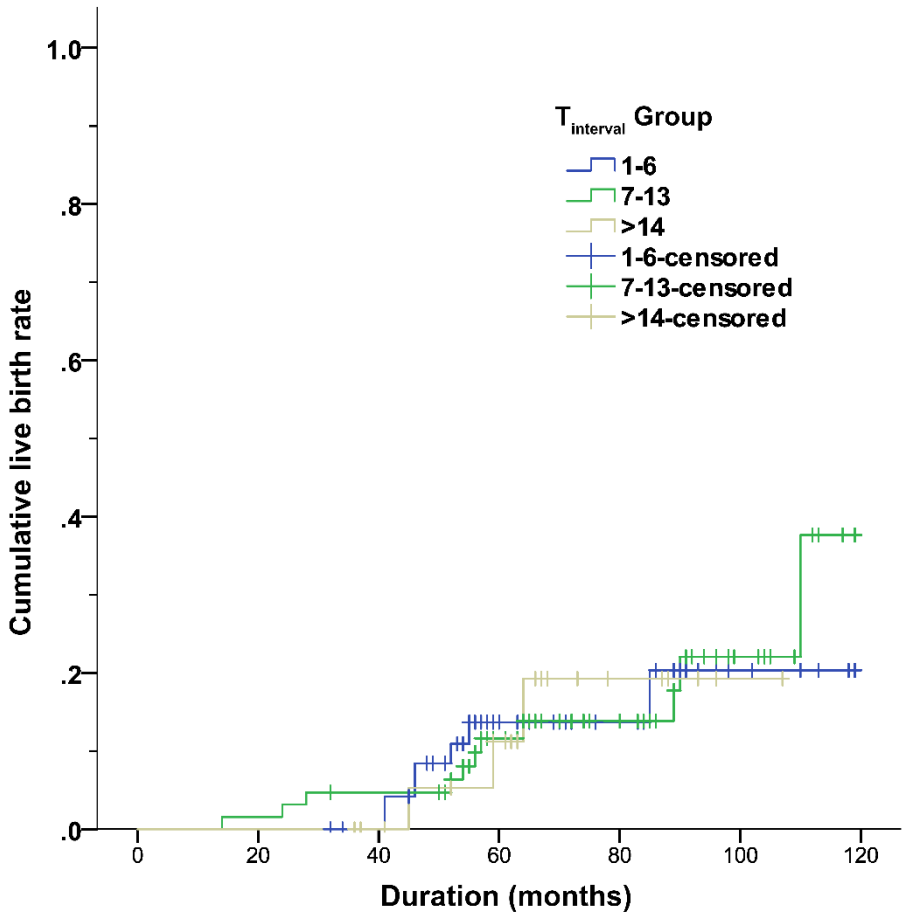


Table 8. Predictors for live birth after chemotherapy

Variables	Live birth	No live birth	*p*-value
Age (years)	31.43 ± 053	33.53 ± 0.56	0.009
$T_{interval}$ (days)	9.10 ± 1.72	10.65 ± 1.52	0.557
Baseline AMH	5.44 ± 0.81	5.08 ± 0.53	0.713
AMH at 12 months (ng/mL)	1.74 ± 0.64	1.33 ± 0.28	0.510
*pcAMH at 12 months	32.66 ± 8.54	25.12 ± 5.46	0.477
$T_{resumption}$ (days)	243.35 ± 42.14	253.13 ± 27.04	0.841
Chemotherapy regimen			0.269†
Anthracycline-based	12 (38.7)	19 (61.3)	
Anthracycline-taxane-based	8 (22.9)	27 (77.1)	
PCO			0.622‡
Yes	12 (26.1)	34 (73.9)	
No	7 (31.8)	15 (68.2)	
Tamoxifen			0.707‡
Yes	14 (30.4)	32 (69.6)	
No	6 (26.1)	17 (73.9)	

Data are shown as mean ± standard error or number (%).

$T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy; AMH = anti-

Müllerian hormone; $T_{resumption}$ = time till resumption of menstruation after chemotherapy; PCO = polycystic ovary.

*pcAMH was calculated by the equation:

$$\text{pcAMH} = \frac{\text{post-chemotherapy AMH value (ng/mL)}}{\text{Baseline AMH value (ng/mL)}} \times 100\ (\%)$$

†Fisher's exact test.

‡Chi-square test.

Table 9. Predictors for AMH ≥ 1 ng/mL at 12 months

Variables	Univariate OR (95% CI)	*p*-value	Multivariate OR (95% CI)	*p*-value
Age (years)	0.83 (0.74-0.92)	<0.001	0.87 (0.77-0.98)	0.024
BMI (kg/m^2)	0.81 (0.70-0.94)	0.006	0.81 (0.68-0.96)	0.013
PCO morphology on ultrasound	4.78 (1.87-12.22)	0.001	3.86 (1.30-11.44)	0.015
$T_{interval}$	1.04 (0.97-1.10)	0.247	.	.
Chemotherapy regimen*	0.79 (0.35-1.78)	0.788	.	.
Tamoxifen use	1.2 (0.46-3.39)	0.670	.	.
Baseline AMH	1.38 (1.17-1.62)	<0.001	. †	. †

AMH = anti-Müllerian hormone; BMI = body mass index; OR = odds ratio; CI = confidence interval; PCO = polycystic ovary; $T_{interval}$ = the interval between the initiation of gonadotropin-releasing hormone agonist and chemotherapy.

*Two group analysis: anthracycline-based chemotherapy versus anthracycline and taxane-based chemotherapy

†Baseline AMH was discarded from multivariate analysis due to multicollinearity with age, BMI, and PCO as shown in Table 3.

IV. Discussion

In this prospective cohort study, we report that changes in AMH level after chemotherapy were not significantly different, regardless of the timing of GnRHa administration. In other words, there was no effect on the ovarian protective effect even if the chemotherapeutic agent was administered during the "flare-up phase" of GnRHa. This suggests that there may be little to no advantage in waiting 1–2 weeks before chemotherapy until pituitary suppression. A possible explanation for this is that although theoretically, the ovarian protective effect relies on pituitary suppression, there may be other direct actions of GnRHa on the ovaries that need to be elucidated (Poggio *et al.*, 2019).

One particular strength of our study is that we used AMH as a surrogate marker to evaluate ovarian function in patients with breast cancer. AMH is considered to be the most valuable marker for ovarian reserve and function, particularly in breast cancer survivors. A large proportion of breast cancer survivors receive either tamoxifen or GnRHa, both of which do not affect plasma AMH levels but may affect other surrogate markers of ovarian function, such as resumption of menses or other female hormones. Therefore, previous studies that evaluated ovarian function using these markers in breast

cancer carry the risk of misclassifying women with declined ovarian function (Seifer and Maclaughlin, 2007).

Although AMH appears to be an ideal marker of ovarian reserve, it can be misleading if PCO is not considered. Women with PCO generally exhibit AMH levels 2 or 3 times higher than the average value at their age, which can be misinterpreted as 2 or 3 times greater ovarian reserve than their actual value (Pellatt *et al.*, 2007). In this study, we demonstrated that there is not only a difference in the absolute value, but also differences in the pattern of change over time compared with baseline AMH values according to the presence of PCO.

Another strength of our study was that we used ranked ANCOVA to compare variables while adjusting for PCO and other factors. The pcAMH data were positively skewed where the mean was greater than the median; thus, a non-parametric analysis was required. With ranked ANCOVA, the confounders were successfully controlled while enabling a non-parametric comparison of variables. In addition, we used pcAMH as the dependent variable to eliminate the need for adjustment for baseline AMH, which may cause multicollinearity issues with age (i.e., adjusted twice).

In the study by Zhang *et al.*, the authors compared sequential versus simultaneous use of chemotherapy and GnRHa in estrogen receptor-positive patients with breast cancer (Zhang *et al.*, 2018). In that study, the main purpose of GnRHa was therapeutic ovarian suppression to prevent cancer recurrence, with the treatment lasting >2 years. There was no difference in the median time to resumption of menstruation between the 2 groups. However, resumption of menstruation was not a suitable marker for that study because all patients received tamoxifen for 5 years which can cause menstrual irregularities, vaginal bleeding, or cessation of menstrual periods. On the other hand, we have the advantage of assessing ovarian function using AMH which is a significantly more reliable marker than resumption of menstruation and in addition, was adjusted for age, BMI, and PCO.

The prevalence of PCO morphology was 32.4%, and it was higher in the group of $T_{interval}$ of 14 days or more. The exact underlying cause of this difference is unclear. However, the prevalence of PCO morphology in general population has not been reported in Korea, and those reported in previous studies using the same criteria used in our study have varied widely. In an Australian study, the prevalence of PCO morphology in the female partners of men with azoospermia was reported to be 23% (Lowe *et al.*, 2005). In another study,

the prevalence of PCO morphology in heterosexual subfertile women was 32%, while the prevalence among lesbian women was 80% of those attending for donor insemination (Agrawal *et al.*, 2004). The prevalence of PCO morphology has been reported to be 54.8% (Escobar-Morreale *et al.*, 2000) and 52.4% (Miyoshi *et al.*, 2013) in women with type 1 diabetes. Further study is needed to determine the prevalence of PCO morphology in general population, and whether the prevalence shown in our study is higher than in the general population.

To our knowledge, this was the third study to use AMH as a surrogate marker for ovarian reserve in patients with breast cancer who received GnRHa for fertility preservation. Moreover, this was the first study to use AMH to compare the effect of different timings of administration GnRHa on ovarian reserve in patients with breast cancer. In a recent study, amenorrhea and AMH was used as a surrogate marker of ovarian reserve. They described the chronological change of AMH, but they did not compare AMH between groups (Zhong *et al.*, 2019). Another previously published study that used AMH as a surrogate maker of ovarian function in patients with breast cancer following chemotherapy and concomitant administration GnRHa for ovarian protection focused on determining factors that influenced or predicted good ovarian reserve (Lee *et al.*, 2018) and reported that tamoxifen was

associated with low serum AMH level at 12 months in the multivariate analysis (adjusted OR 0.156 [95% CI 0.032–0.766]). In previous studies, tamoxifen was not associated with decreased AMH levels, and some studies reported associations with high AMH levels (Shandley *et al.*, 2017, Dezellus *et al.*, 2017, Anderson *et al.*, 2017). The authors addressed the issue in the discussion but did not describe the reason. A possible explanation is that they did not take PCO into account. As shown in our study, PCO is significantly associated with not only increase in the absolute AMH value but also an increase in the rate of AMH recovery relative to baseline AMH. In our multivariate analysis, young age, low BMI, and the presence of PCO were associated with high AMH levels at 12 months. High baseline AMH level was undoubtedly associated with high AMH level at 12 months; however, it was discarded from multivariate analysis due to multicollinearity (Table 2). Low BMI was associated with high AMH after chemotherapy in our study. Although the exact mechanism underlying this is unclear, it is consistent with a previous study that obesity adversely affects serum AMH when adjusted for PCO (Moy *et al.*, 2015).

The high pcAMH found in PCO group at 36 months (60.68 ± 12.88%) may have been partially due to the presence of two patients that showed pcAMH values of 122.05% and

125.35%. Elevation of AMH higher than baseline after chemotherapy has not been reported before, and the underlying mechanism, or the clinical significance of this phenomenon needs further research.

Tamoxifen use for women who had a high level of estrogen receptor protein (ER) measured in their primary tumor, it is known that the improvement of recurrence was greater during the first 5 years, and the improvement in survival was steady throughout the first 10 years (Early Breast Cancer Trialists' Collaborative Group, 1998). In trials, participants typically used risk-reducing medication for 3-5 years (Nelson *et al.*, 2019). Since tamoxifen is known to be related to high risk of congenital malformations, ceasing tamoxifen is highly advised before the attempt to conceive (Halakivi-Clarke *et al.*, 2000, Braems *et al.*, 2011). For this reason, the patients taking tamoxifen would not be able to conceive.

The analysis of delivery is very meaningful for several reasons. The reproductive outcome of our study showed that 30.4% of married women delivered after chemotherapy. Based on our data, physicians can expect above-mentioned proportion of patients would try to conceive, and mostly between 4-7 years of follow up. The patients who delivered would have had to balance between continuation of anticancer

therapy and the desire to get pregnant. Time-till-delivery can thus reflect the result of those longtime struggle, similar to the study published earlier in the analysis of frozen embryo transfer (Shin *et al.*, 2018).

However, there are some limitations to this approach because of various situations. For instance, the risk of recurrence may have been too high to cease tamoxifen medication to try to conceive. Not all patients desired fertility, and not all women who desired fertility got married. Last but not least, in women who eventually delivered, it is unknown how they became pregnant (e.g. natural or assisted reproductive technique), or how long they have tried to become pregnant.

The other weakness of our study was that the efficacy of the GnRHa was not assessed by comparing with patients who did not receive GnRHa. However, because our cohort included only patients who received GnRHa, acquiring data from patients with breast cancer who did not receive GnRHa would require another study protocol, which was beyond the scope of the present investigation.

An important consideration to keep in mind when interpreting our results is that the composition of our cohort may be different from the general young breast cancer

population. These were women diagnosed with breast cancer but were selectively referred to the fertility clinic for fertility preservation. Patients with severe disease who required prompt initiation of chemotherapy may not have been included in the cohort. Furthermore, the reason that there was only one patient who received CMF is that it is known for having a higher risk for ovarian failure than other regimens, and medical oncologists generally avoid this regimen in women having a significant interest in preserving fertility.

V. Conclusion

In conclusion, regardless of the timing of administration before chemotherapy, there was no difference in the efficacy of the GnRHa in preserving the ovarian reserve assessed using AMH and live birth as a surrogate marker in patients with breast cancer.

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국문 초록

배경: 보조항암화학요법은 난소의 예비능을 감소시켜 향후 임신의 가능성을 낮추고, 폐경을 앞당기는 부작용이 발생할 위험이 높다. 이에 대한 대비책으로 난소의 기능을 억제하는 약물을 투약하여 보조항암화학요법으로 기인하는 난소기능 저하를 방지하고자 하는 전략이 점차 널리 이용되고 있다. 이러한 전략에 사용되는 약제가 생식샘자극호르몬분비호르몬작용제이며, 이 약제는 투약 초기 수 일간 생식샘자극호르몬의 분비를 오히려 증가시키는 'flare-up' 현상을 특징으로 한다. 난소의 기능이 억제되지 않고 오히려 항진된 이 시기에 보조항암화학요법을 시작한 경우 난소의 보호 효과에 대해서는 현재까지 알려진 바가 없다. 본 연구의 목적은 유방암 환자에서 보조항암화학요법과 병용 투여한 생식샘자극호르몬분비호르몬작용제의 투여 시작 시점에 따른 난소 보호 효과의 차이를 파악하는 데에 있다.

방법: 서울대학교병원에서 2009년 10월부터 2016년 2월까지 유방암으로 진단받고 보조항암화학요법을 시행 받았으며, 가임력 보존을 위해 생식샘자극호르몬분비호르몬작용제를 항암제와 병용 투여한 40세 이하의 환자를 대상으로 하는 전향적 관찰적 코호트 연구이다. 난소 기능 평가를 위해 보조항암화학요법 전(기저치) 및 보조항암화학요법 종료 후에 정기적으로 검사한 항뮬러관호르몬의 혈중농도를 난소 기능의 지표로 이용하였다. 생식샘자극호르몬분비호르몬작용제의 투여 후 몇 일 후에 보조항암화학요법을 시작하였는지에 따라 대상 환자들을 1-6일, 7-13일, 14일 이상의 세 군으로 나누어 분석하였다. 항뮬러관호르몬 혈중농도 기저치 대비 항암화학요법 종료 후 항뮬러관호르몬 혈중농도

변화를 백분율로 환산하여 pcAMH라는 지표를 정의하여 세 군간의 비교를 시행하였다. 순위기반 공분산분석을 이용하여 나이, 비만도, 초음파상 다낭성난소의 유무를 보정하여 통계학적 분석을 시행하였다. 이에 더하여, 보조항암화학요법 종료 후 분만한 여성의 수를 세 군간 비교하였으며, 항암치료 종료 후 12개월에 항뮬러관호르몬의 혈중농도가 1 ng/mL 이상인 것과 관련된 인자들을 파악하기 위하여 다변수분석을 시행하였다.

결과: 대상 환자들의 나이의 중위값은 32세였다. 항뮬러관호르몬의 기저치는 세 군 간에 유의한 차이를 보이지 않았다 (평균 ± 표준오차, 5.0 ± 0.4 ng/ml [1–6일], 5.3 ± 0.7 ng/ml [7–13일], and 8.1 ± 1.3 ng/ml [≥14일 이상], p = 0.25). 항암화학요법 종료 후 pcAMH의 세 군 간 비교에서 각 평가 시점 별 p 값은 3개월 0.33, 6개월 0.73, 12개월 0.83, 24개월 0.15, 36개월 0.39로 나타났으며, 통계적으로 유의한 차이를 보이지 않았다. 혼인관계가 확인된 69명의 환자 중 총 21명의 여자가 보조항암화학요법 종료 후 생아를 출산하였다 (30.4%). 생아 출산한 여성의 비율의 비교에서 세 군 간에 유의한 차이를 보이지 않았으며(p = 0.680), 카플란-마이어 곡선 및 로그-랭크 테스트를 이용한 세 군간의 비교에서도 유의한 차이를 보이지 않았다(p = 0.999). 한편 다변수분석에서 항암화학요법 종료 후 12개월 시점에 항뮬러관호르몬 혈중농도 1 ng/mL이상의 예측인자는 젊은 나이 (p = 0.024), 낮은 비만도 (p = 0.013), 초음파상 다낭성난소가 존재하는 경우 (p = 0.015)로 확인되었다.

결론: 가임력보존 목적으로 생식샘자극호르몬분비호르몬작용제를 유방암 환자에게 보조항함화학요법과 병용투여한 경우, 생식샘자극호르몬분비 호르몬작용제의 투여 시점에 따른 난소의 보호 효과의 유의한 차이가

관찰되지 않았다. 본 연구의 결과에 따르면 생식샘자극호르몬분비호르몬 작용제의 투약 초기 'flare-up' 시기에 투약한 경우에도 난소의 보호효과에는 차이가 없었다.

주요어: 가임력보존, 다낭성난소, 보조항암화학요법, 생식샘자극호르몬 분비호르몬작용제, 유방암, 항뮬러관호르몬

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