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The Association of Parity and Breastfeeding with Anti-Müllerian Hormone Levels at Two Time Points

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Contributors

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Bernard A. Rosner contributed to analysis and interpretation of data and critical revision of the manuscript.
Elizabeth R. Bertone-Johnson contributed to conception and design of the study, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, obtaining funding, and supervision.

Declaration of competing interests

The authors declare that they have no competing interests.

Ethical approval

The study protocol was approved by the Institutional Review Board at Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health in Boston, MA.

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Abstract

Objective: To evaluate the association between parity and breastfeeding and anti-Müllerian hormone levels (AMH) and change in AMH levels over time. Furthermore, we examined whether AMH levels mediate the relation of parity and breastfeeding with age at menopause.

Study design: Observational, prospective cohort study.

Main outcome measures: AMH levels were assessed in a subset of premenopausal participants in the Nurses' Health Study II, including 1619 women who provided a blood sample in 1996–1999 and an additional 800 women who provided a second premenopausal sample in 2010–2012.

Results: In multivariable linear regression models adjusted for parity, body mass index, smoking, and other factors, mean log AMH levels in 1996–99 were 39% higher in women reporting ≥25 months of total breastfeeding vs. <1 month (P for trend = 0.009). Parity was not associated with AMH levels after adjustment for breastfeeding. Neither parity nor breastfeeding was associated with decline in AMH levels over 10–12 years. Breastfeeding duration was positively associated with age at menopause (*P* for trend = 0.01), with evidence that the association was mediated via AMH.

Conclusions: Our results suggest that breastfeeding is associated with higher AMH levels and later onset of menopause, and support the hypothesis that observed relations of parity with AMH levels and menopause timing may be largely attributable to breastfeeding.

Keywords

AMH; anti-Mullerian hormone; parity; breastfeeding; women's health

1. Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein and a member of the transforming growth factor-beta family [1]. In women, AMH is produced by the granulosa cells of the preantral and small antral follicles in the ovaries [2]. AMH plays a key role in regulating folliculogenesis by inhibiting the initiation of primordial follicle recruitment and the sensitivity to follicle-stimulating hormone (FSH) for growing follicles [3, 4]. AMH is measured in serum or plasma and secreted in proportion to the number of developing follicles [1, 2, 5–7].

Over a woman's reproductive life, AMH levels peak during late puberty, decline after mid-twenties, and become undetectable prior to menopause. Because circulating levels are correlated with the size of the ovarian follicle pool, AMH is used as a clinical measure of ovarian aging [1, 8]. Furthermore, population-based studies have shown that AMH strongly predicts onset of menopause independent of chronologic age in reproductive-aged women

[9–14]. Higher AMH levels have also been associated with risk of early menopause among healthy women [15].

Previous studies suggest that parity and breastfeeding are related to timing of menopause [16–23]. In a recent analysis conducted in the Nurses' Health Study II (NHS2), we observed inverse associations of both factors with risk of early natural menopause [24]. Findings suggest that reproductive factors such as parity and breastfeeding may delay onset of menopause, perhaps via ovulation suppression and slowing depletion of the follicle pool, which may in turn influence AMH levels. However, few studies have evaluated the relationship between reproductive factors and AMH, and findings have been inconsistent. While two cross-sectional studies observed higher AMH levels in women with higher parity [25, 26], others have reported no association or an inverse association with parity [27, 28]. Few studies have assessed breastfeeding in relation to AMH levels [28]. To our knowledge, no study has prospectively evaluated whether parity and breastfeeding are associated with AMH levels and how these variables are associated with decline in AMH over time. Because parity is inherently related to opportunity for breastfeeding, it is important for research to consider these reproductive factors jointly. It is also unknown whether duration of exclusive breastfeeding may be more strongly related to AMH levels than any breastfeeding (i.e., breastfeeding occurring along with supplemental foods), as exclusive breastfeeding is more likely to suppress ovulation. These are important questions to address to better understand the potential pathway(s) by which reproductive events affect timing of menopause.

We examined the association between pregnancy and breastfeeding with AMH levels and change in AMH levels over 11 to 15 years among a subset of women in the NHS2. We further investigated whether AMH levels may mediate a relationship of pregnancy and breastfeeding with age at menopause.

2. Methods

2.1. Study population

NHS2 is a prospective study of 116,429 US female registered nurses who responded to a mailed questionnaire in 1989. Participants were aged 25–42 at baseline and provided information on health-related behaviors and medical conditions, such as oral contraceptives (OC), pregnancy history, and smoking status. Data was collected biennially since 1989 through mailed questionnaires up until 2015; an average response rate has been 94%. The study protocol was approved by the Institutional Review Board at Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health in Boston, MA.

2.1.1. NHS2 blood cohort—Participants of the NHS2 cohort who had not been diagnosed with cancer other than non-melanoma skin cancer were invited to provide blood samples between 1996 and 1999. Women who were premenopausal, not using hormone therapy (HT) or oral contraceptives (OC)s, and who had not been pregnant in the past 6 months were asked to collect one timed sample in the follicular (day 3 – 5) and one timed sample in the luteal phase (an estimated 7 – 9 days before the anticipated start date of next menses). Women with irregular cycles were asked to collect their luteal phase sample 22 days after last menses. Women who were premenopausal and using HT or OCs were asked

to provide a single untimed sample. The start date of the next menses was confirmed by a postcard, by which timing of the luteal phase sample for each participant was determined. Samples were centrifuged, separated, and archived at < −130°C in monitored nitrogen freezers. During this time, samples were received from 29,611 women of whom 23,000 were premenopausal, aged 32–54. Women who provided a blood sample in 1996–1999 were invited to provide a second sample in 2010–2012, following similar procedures. Samples were received from 16,510 women and approximately 4,000 were still premenopausal at time of second blood collection.

2.2. Assessment of menopause timing and sample selection

Menopause status and timing was assessed prospectively in the NHS2 as follows. On each biennial questionnaire, participants were asked if their menstrual periods had stopped permanently. Women had to select one of the following options: 1) No, premenopausal; 2) Yes, no menstrual periods; 3) Yes, had menopause but now have periods induced by hormones; and 4) Not sure (e.g., started hormones prior to cessation periods). Women who reported that their periods had stopped were then asked: 1) at what age did your periods cease, and 2) what reason did your periods cease (i.e., surgery, radiation or chemotherapy, or natural). Furthermore, women were asked about menopausal HT use including timing and type. Age at menopause was defined as age after 12 consecutive months of amenorrhea. For a small number of women who reported being postmenopausal on one questionnaire and then subsequently reported being premenopausal, their age at menopause was documented as age after which periods were absent for 12 months or more and persisted for at least three consecutive questionnaires.

We selected the following groups of women for analysis: women whose premenopausal blood samples were assayed as part of an early menopause nested case-control study [15] and an additional group of women who contributed premenopausal samples at both blood collections (supplemental figure). Briefly, we selected n=327 women who reported natural menopause between date of receipt of their blood sample and age 45. Controls were n=491 women who reported menopause after age 45, and included 327 women with menopause at age ≥ 47 years matched 1:1 with cases on age at blood draw ((± 4 months), fasting status, time of day, season of blood collection, and sample type (luteal phase or random timing).

Secondly, we identified 800 women providing blood samples in both 1996–99 and 2010–2012, who were premenopausal at both time periods. These women were selected to evaluate how change in AMH over 11 to 15 years was associated with timing of menopause.

2.3. Assessment of reproductive factors

Information on parity and breastfeeding was obtained from questionnaires at baseline and updated every two years until each participant's time of blood collection. On each biennial questionnaire, women were asked whether they had a pregnancy within the past two years and the number of pregnancies that lasted at least six months. In 2009, total pregnancy history was verified by asking participants to report the calendar year each pregnancy ended.

Participants were also asked to report the number of months in total they breastfed for all their births combined. Participants specified whether they did not breastfeed, had no

children, or could not remember, which was used to estimate cumulative total breastfeeding. In 1997 and 2003, women who breastfed for at least 1 month reported when they started to give formula or purchased milk at least once daily, started giving solid food at least once daily, started pumping breast milk at least 4 days per week, and went at least 6 hours at night without breastfeeding. Detailed information was collected on each pregnancy for women who indicated they breastfed for up to 4 children. Women with more than 4 children also reported total additional months of breastfeeding. Based on these responses, we defined cumulative exclusive breastfeeding as total months a woman fed their infants with breast milk only and no other liquids or solids.

2.4. Assessment of anti-Müllerian hormone

AMH (ng/mL) was measured by an ultra-sensitive ELISA assay from ANSH Labs (picoAMH, Webster, TX) employing a quantitative sandwich enzyme immunoassay technique in the laboratory of Dr. Nader Rifai, Children's Hospital, Boston, MA. Samples from the early menopause nested case-control study (n=819) were randomly ordered and assayed together in the same box in July 2015. Paired premenopausal samples (n=800) were measured by the same assay (picoAMH) in the Rifai laboratory between January and March 2018, with pairs randomly ordered and assayed together in the same box. For each assay, blinded samples from a plasma pool, equaling n = 10% of analytic samples, were randomly distributed across boxes. The coefficient of variation (CV) for the first and second set of samples were 8.6% and 17.4%, respectively. AMH levels below the limit of detection were replaced by a value of LOD/√2 (0.0043 ng/mL) [29]. Approximately 16.0% of paired samples had values below the limit of detection.

2.5. Assessment of covariates

At baseline in 1989, women provided information on race/ethnicity, height, weight at age 18, age at menarche, and the number of years until menstrual cycle became regular after menarche. At time of blood collection, information was collected on current weight, alcohol use, exogenous hormone use, physical activity, and smoking status. Pack-years of cigarette smoking, duration of OC use, and infertility were measured using the biennial questionnaire closest in time to each individual's date of blood collection. Characteristics related to blood sample collection were also reported, including fasting status and time of blood collection, which were used to derive season at blood collection and luteal day.

2.6. Statistical analysis

AMH values were log transformed for analysis to meet the assumption of normality. We first assessed the relations of covariates at the time of blood collection with age-adjusted AMH levels in 1996–1999 using generalized linear models in analytic cohorts comprised of: 1) all participants; 2) all controls; 3) and women with paired samples only. While mean AMH level differed between groups, we observed minimal differences in the associations between AMH and covariates across groups. Thus opted to use all available data to maximize power and generalizability. One participant who had outlier values for AMH was excluded from the analysis. Model estimates based on log transformed AMH were exponentiated for interpretability as geometric means (GM).

The relations of parity, cumulative total breastfeeding, and cumulative exclusive breastfeeding with log AMH levels at first blood collection were evaluated using linear regression to estimate mean differences and 95% confidence intervals (CI). Furthermore, we estimated mean decline of log AMH levels, defined as the difference of log AMH levels from first to second blood collection, among the subset of women with paired blood samples. Linear trends were assessed and tested by modeling each reproductive factor as a continuous variable. In linear models of log transformed AMH, exponentiated coefficients can be interpreted as percent differences (i.e., exp(b)−1=% change).

Our first multivariable (MV) model was adjusted for age (age and age^2) and assay characteristics (i.e., fasting status, time of day, and luteal day). In a second MV model, we further adjusted for BMI, pack years of cigarette smoking, smoking status, exogenous hormone use, duration of OC use, years until menstrual cycle became regular after menarche, history of infertility, age at menarche, and alcohol intake. In a third MV model, we assessed independent associations of parity and breastfeeding with log AMH levels by mutually adjusting for each reproductive factor. When evaluating decline of log AMH levels, AMH at first blood collection was included in the minimally adjusted model.

Finally, we evaluated associations of age at menopause with parity and breastfeeding, and the role of AMH in these associations among 1,389 women who had reached menopause by the 2015 questionnaire cycle. To evaluate AMH as a mediator of the relation of parity and breastfeeding with age at menopause, we ran separate models further adjusting for AMH levels at first blood collection among all participants. In addition, the proportion of the total effect attributable to AMH was determined by estimating direct and indirect effects of each reproductive factor. Statistical tests were two-sided with *P* values <0.05. All analyses were conducted with SAS 9.4 (SAS Institute, Cary, NC).

3. Results

Age and age-adjusted characteristics of participants and at first blood collection are shown in Table 1, along with geometric mean AMH levels (ng/mL). The mean age at first blood collection was 39 years (range, 32–46). Geometric mean AMH levels were highest among women who experienced menarche at an older age, had a longer duration until menstrual cycle regularity, never smoked, never used oral contraceptives, or had no history of infertility.

We examined the mean differences of log AMH levels by categories of parity and breastfeeding (Table 2). In MV1, higher parity was significantly, positively associated with higher AMH levels, both based on comparisons of categories of parity as well as test for linear trend. After adjusting for covariates (MV2), associations were slightly attenuated, but followed a similar pattern and remained statistically significant. For example, compared to nulliparous women, mean log AMH levels in women with ≥ 3 pregnancies were 0.27 higher (95% CI, 0.07 – 0.47; *P* for trend = 0.02), equivalent to a difference of 31%. However, after additional adjustment for cumulative duration of total breastfeeding (MV3), parity was no longer linearly associated with AMH levels (*P* for trend = 0.82).

Longer cumulative duration of total breastfeeding was associated with higher AMH levels in MV1 and MV2 (Table 2). Further adjustment for parity (MV3) only modestly attenuated findings and the relationship between AMH and cumulative duration of total breastfeeding remained statistically significant. For example, compared to women reporting <1 month of breastfeeding, mean log AMH was 0.33 higher (approximately 39% higher) in those reporting ≥25 months (95% CI, 0.08 – 0.58; *P* for trend = 0.009). Cumulative duration of exclusive breastfeeding was also positively associated with AMH levels in the minimally and fully adjusted model that also accounted for parity (*P* for trend = 0.008). For each duration category, model estimates for exclusive breastfeeding were slightly higher than for total breastfeeding.

The median duration between first and second blood collection was 13.3 years (IQR, 12.8 – 13.8 years), with a mean age at second blood collection of 51 years (standard error = 0.09; range, 46–60). Mean difference in AMH levels between blood collections was 4.82 ng/mL. AMH levels in 2010 – 2012 were very low (0.23 ng/mL), with modest variability (standard deviation = 0.56). We did not observe a clear relation between parity or breastfeeding and the amount AMH levels declined over time (Table 3). In fully adjusted models, level of decline was greatest for women reporting 1 pregnancy and women reporting moderate levels of breastfeeding, with no evidence of linear trend (P=0.41 and P=0.91). When restricting our analyses to women without a history of infertility (n=642), results were similar to our main findings (data not shown).

The relation of parity and breastfeeding with age at menopause and assessment of the role of AMH in these associations is shown in Table 4. In our main MV model, cumulative duration of total and exclusive breastfeeding were linearly associated with age at menopause. For example, the mean age at menopause for women reporting ≥25 months of total breastfeeding was more than 1 year later than that for women reporting <1 month (50.8 vs. 49.6 years; *P* for trend = 0.002). After adjustment for AMH levels at first blood collection (MV2), no differences in age at menopause were observed between categories of breastfeeding duration, suggesting that AMH contributed substantially to these associations. In fact, the proportion mediated by AMH was estimated to be 76% (95% CI: 34 – 95%) for cumulative duration of total breastfeeding and 84% (95% CI: 20–99%) for cumulative duration of exclusive breastfeeding. In contrast, parity was not associated with age at menopause.

4. Discussion

In this study, we observed that a longer duration of breastfeeding was associated with higher AMH levels even when accounting for parity. In contrast, higher parity was associated with higher AMH levels, but not after adjusting for breastfeeding. Furthermore, we found higher mean age at menopause with higher cumulative duration of total and exclusive breastfeeding, but not after adjustment for AMH, providing evidence that this relation is largely mediated by AMH.

It is hypothesized that pregnancy inhibits the growth and apoptosis of antral follicles in the ovaries [25]. In addition, breastfeeding inhibits the pulsatile secretion of gonadotropin-releasing hormone from the hypothalamus by elevating levels of prolactin. This disrupts

the hypothalamic-pituitary-ovary axis, which prevents the secretion of FSH and luteinizing hormone [30]. Furthermore, exclusive breastfeeding prolongs postpartum return to ovulation due to the high frequency, intensity, and duration of breast milk production; in contrast, non-exclusive breastfeeding does not have the same effect on ovulation [30]. The prolonged anovulatory periods related to these reproductive factors may slow the depletion of the ovarian follicle pool and the rate of oocyte loss [26, 27, 31, 32], in turn influencing AMH concentration and delaying menopause.

Ours is among the first studies to concurrently examine how parity and breastfeeding are associated with AMH, change in AMH over time, and menopause timing, in order to disentangle the complex relationship between these factors. In a recent analysis in the NHS2 cohort, parity was inversely associated with risk of early menopause, but results were considerably attenuated after adjustment for breastfeeding, though still significant [24]. Breastfeeding was independently associated with risk of early menopause, irrespective of parity. Furthermore, women who breastfed exclusively for 7 to 12 months had the lowest risk. These findings suggest that some of the lower risk of early natural menopause previously attributed to parity in observational studies could actually be attributable to breastfeeding. Our present results are consistent with these findings, in that breastfeeding was more strongly associated with AMH and timing of menopause than parity in models adjusting for both simultaneously. We also observed slightly stronger associations of AMH with exclusive than total breastfeeding.

Our relatively null findings regarding parity and AMH are also similar to other studies. For example, a study by Jung et al. utilized data from 671 women from nine cohorts and found no relationship between parity and AMH levels [27]. In that study, the adjusted median (IQR) AMH was 1.05 ng/mL (0.33 – 1.73) for nulliparous and 0.95 ng/mL (0.42–2.00) for parous women, and there was no evidence of linear trend. A cross-sectional study comprised of 2,320 women reported that parity was associated with age-specific AMH levels, but not after adjusting for OC use, cycle irregularity, smoking [26]. In another cross-sectional study of 186 participants, mean AMH levels were higher among parous (3.54 ng/mL) than nulliparous women (2.53 pg/mL; $p<0.001$) when controlling for age at menarche and duration of menstrual cycle [25]. The authors noted that women who were multiparous also had higher ovarian volume and antral follicle count. Nevertheless, this study did not evaluate the effect of breastfeeding; therefore, it is not clear whether associations observed with parity could have been attributed to breastfeeding.

To our knowledge, only one other study has evaluated the relation of breastfeeding with AMH levels [28]. Whitworth et al. assessed AMH levels among 425 rural South African women aged 22–26 and found no significant difference in AMH levels among women who breastfed 18–27 months or ≥28 months compared to women who breastfed 0–17 months, nor among women who were currently breastfeeding versus those who were not breastfeeding. The substantial difference in age between their population and ours makes comparisons of findings difficult. Further research in large cohorts of women of a range of reproductive ages will be important for better understanding the relation of breastfeeding with ovarian reserve and AMH. In our study, neither parity nor breastfeeding were associated with level of decline in AMH levels over approximately 13 years. Of note,

the inter-individual variation of AMH levels at second blood collection was very low as many participants were close to menopause, and AMH has been observed to decline to near undetectable levels by this time [33]. The late timing of this second measurement makes it difficult to infer whether these reproductive events impact AMH trajectories over time. In our analysis, the decline in AMH is assumed to be linear; however, previous research has shown that the trajectory is non-linear during certain periods of life. Additional studies utilizing longitudinal measurements of AMH at younger ages during the mid-late reproductive period are required to better understand these relations.

Our study has several potential limitations. We relied on self-reported breastfeeding history and duration, which could result in misclassification. However, validation studies have shown that recall of breastfeeding several years after a child's birth is relatively accurate [34, 35]. In addition, breastfeeding history was assessed prospectively and updated with detailed information on duration of breastfeeding for each child and pregnancy history. Therefore, misclassification of breastfeeding would likely have minimal impact on the observed association. Furthermore, though we relied on self-reported onset of menopause, the potential for misclassification was minimized several ways. At blood collection, participants reported menstrual cycle regularity and confirmed they were premenopausal. Menopause status was also queried prospectively every 2 years until end of follow-up. In an analysis in the Nurses' Health Study, 82% of women were able to recall their age at menopause within one year on subsequent questionnaires [36].

The prevalence of early menopause (menopause before age 45) was slightly higher in our sample than in the general population, which could potentially impact the generalizability of our findings. However, we did not find the association of demographic, reproductive and behavioral factors with AMH levels to vary between cases, controls and randomly chosen women with paired samples, which suggests that relations are generally robust. Lastly, our study population is predominantly White, and results may not be generalizable to other racial/ethnic groups; however, we do not expect the physiologic associations between reproductive factors, AMH levels, and menopause timing to vary by race/ethnicity. Nonetheless, additional prospective studies are warranted to investigate these relationships in diverse populations.

In conclusion, we observed a positive association of breastfeeding with AMH levels and later onset of menopause. Our results also support the hypothesis that previously observed associations of parity with menopause timing may be largely attributable to breastfeeding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Anti-Müllerian hormone levels are strongly related to menopause timing.
- Whether parity and breastfeeding are related to anti-Müllerian hormone is unknown.
- We found breastfeeding to be positively associated with anti-Müllerian hormone.
- Parity was not associated with anti-Müllerian hormone after adjustment.
- Breastfeeding may largely explain observed relations of parity and menopause timing.

Table 1.

Age and Age-adjusted[a] characteristics of all participants (n=1618) at first blood collection in 1996–1999, Nurses' Health Study 2.

		N (%)	AMH (ng/ml), Geometric Mean (SE)
Age (years)			
	30–34	65 (4.0)	2.5 (0.19)
	35–39	803 (49.6)	2.1 (0.05)
	40–44	703 (43.4)	0.7 (0.06)
	>45	47 (2.9)	0.2 (0.22)
White race			
	No	59 (3.6)	1.1 (0.20)
	Yes	1559 (96.3)	1.3 (0.04)
Non-Hispanic ethnicity			
	No	27 (1.7)	1.0 (0.29)
	Yes	1591 (98.3)	1.2 (0.04)
Body mass index (kg/m^2)			
	<18.5	33 (2.0)	1.5 (0.26)
	18.5– <25	996 (61.5)	1.3 (0.05)
	25 – <30	358 (22.1)	1.2 (0.08)
	≥ 30	228 (14.1)	1.0 (0.10)
Body mass index at age 18 (kg/m^2)			
	<18.5	220 (13.6)	1.4 (0.10)
	18.5– <25	1250 (77.2)	1.2 (0.04)
	25 – <30	108 (6.7)	1.1 (0.14)
	≥ 30	31 (1.9)	1.1 (0.27)
Age at menarche (yrs.)			
	9 – 11	335 (20.7)	1.0 (0.08)
	12	508 (31.3)	1.2 (0.07)
	13	454 (28.0)	1.4 (0.07)
	14–17	321 (19.8)	1.3 (0.08)
Years until menstrual cycle became regular (yrs.) [b]			
	<1	765 (47.2)	1.0 (0.05)
	1 – 2	387 (23.9)	1.1 (0.07)
	3 – 4	131 (8.1)	1.3 (0.13)
	≥5	154 (9.5)	1.5 (0.12)
Smoking status			
	Never	1166 (72.1)	1.3 (0.04)
	Former	326 (20.1)	1.2 (0.08)
	Current	126 (7.8)	0.7 (0.13)
Pack years of cigarette smoking			
	None	1166 (72.1)	1.3 (0.04)

	N (%)	AMH (ng/ml), Geometric Mean (SE)
1–5	169 (10.4)	1.5 (0.11)
6–10	111 (6.9)	1.0 (0.14)
11–15	80 (4.9)	0.7 (0.17)
16–20	38 (2.3)	0.9 (0.24)
≥21	53 (3.3)	0.5 (0.21)
Alcohol intake		
None	561 (34.7)	1.3 (0.06)
≤5–6 drinks per week	949 (58.6)	1.2 (0.05)
1 drink per day	59 (3.6)	1.5 (0.20)
2– >6 drinks per day	49 (3.0)	1.1 (0.22)
Physical activity (days per week)		
<1	532 (32.9)	1.2 (0.06)
1	371 (22.9)	1.3 (0.08)
2 – 3	483 (29.8)	1.2 (0.07)
≥4	232 (14.3)	1.4 (0.10)
Duration of oral contraceptive use (mo.)		
Never	306 (18.9)	1.5 (0.09)
1–23	318 (19.6)	1.3 (0.08)
24–71	528 (32.6)	1.2 (0.06)
72–119	268 (16.7)	1.1 (0.09)
≥120	173 (10.7)	0.9 (0.11)
Current exogenous hormone use		
No	1590 (98.3)	1.3 (0.04)
Yes	28 (1.7)	0.2 (0.28)
History of infertility		
None	1258 (77.8)	1.3 (0.04)
Ovulatory	121 (7.4)	1.3 (0.14)
Other[c]	238 (14.7)	0.9 (0.10)

[a] AMH levels were adjusted for age and age^2

[b] Percent sum does not equal 100 because participants whose menstrual cycle never became regular are not presented

[c] Other causes for infertility include tubal blockage, endometriosis, cervical mucous factors, spouse/partner, infertility not investigated or unknown, other cause of infertility

Table 2.

Mean differences (95% CIs) of log AMH levels in 1996–99, by reproductive factors (n=1618), Nurses' Health Study 2.

Reproductive Factor	No. of Participants	MV1		MV2		MV3	
		β	95% CI	β	95% CI	β	95% CI
Parity							
0	310	REF	--	REF	--	REF	--
1	261	0.24	(0.002, 0.48)	0.16	(−0.06, 0.39)	0.11	(−0.12, 0.34)
2	595	0.33	(0.13, 0.53)	0.22	(0.03, 0.42)	0.13	(−0.08, 0.34)
≥ 3	451	0.42	(0.21, 0.64)	0.27	(0.07, 0.47)	0.11	(−0.12, 0.36)
Per pregnancy		0.10		0.06		0.01	
		p-trend =0.0005		*p*-trend = 0.02		*p*-trend = 0.82	
Cumulative total breastfeeding duration (mo.)							
0 – <1	436	REF	--	REF	--	REF	--
1 – 6	176	0.08	(−0.17, 0.33)	−0.004	(−0.24, 0.23)	−0.02	(−0.27, 0.24)
7 – 12	240	0.19	(−0.03, 0.42)	0.09	(−0.13, 0.30)	0.07	(−0.16, 0.31)
13 – 18	225	0.36	(0.13, 0.60)	0.18	(−0.04, 0.40)	0.17	(−0.08, 0.41)
19 – 24	158	0.34	(0.08, 0.61)	0.11	(−0.14,0.36)	0.09	(−0.20, 0.38)
≥25	369	0.55	(0.35, 0.75)	0.35	(0.16, 0.55)	0.33	(0.08, 0.58)
Per month		0.01		0.01		0.01	
		p-trend <0.0001		*p*-trend = 0.0006		*p*-trend = 0.009	
Cumulative exclusive breastfeeding duration (mo.)							
0 – <1	707	REF	--	REF	--	REF	--
1 – 6	289	0.23	(0.03, 0.43)	0.12	(−0.07, 0.30)	0.09	(−0.10, 0.29)
7 – 12	345	0.32	(0.13, 0.51)	0.18	(0.01, 0.36)	0.16	(−0.03, 0.34)
13 – 18	171	0.53	(0.29, 0.78)	0.38	(0.15, 0.61)	0.34	(0.10, 0.58)
≥19	70	0.36	(0.006, 0.72)	0.26	(−0.07, 0.59)	0.20	(−0.17, 0.56)
Per month		0.02		0.02		0.02	
		p-trend <0.0001		*p*-trend = 0.0005		*p*-trend = 0.008	

MV1: adjusted for age at blood collection (age, age^2) and assay characteristics (fasting status [dichotomous], time of blood collection [4 categories], season of blood collection [winter, spring, fall, summer], luteal day [4 categories])

MV2: MV1 + further adjusted for pack-years of smoking (continuous), smoking status (never, current, former), BMI (continuous), exogenous hormone use (dichotomous), duration of oral contraceptive use (never, 1–23, 24–71, 72–119, ≥120 months), years until cycle became regular (never, <1, 1 – 2, 3 – 4, ≥5 years), infertility history (dichotomous), age at menarche (≤11, 12, ≥13 years), and alcohol intake (never, ≤6 drinks per week, 1 drink per day, ≥2 drinks per day)

MV3: MV2 + further adjusted for other reproductive factor: cumulative total breastfeeding duration (continuous) or parity (continuous)

Table 3.

Mean decline in log AMH levels over 13–15 years by reproductive factors (n=799), Nurses' Health Study 2.

Reproductive Factor	No. of Participants	MV1		MV2		MV3	
		β	95% CI	β	95% CI	β	95% CI
Parity							
0	114	REF	--	REF	--	REF	--
1	146	0.44	(0.11, 0.77)	0.45	(0.12, 0.79)	0.45	(0.10, 0.80)
2	307	0.23	(−0.06, 0.52)	0.30	(−0.002, 0.60)	0.31	(−0.02, 0.63)
≥ 3	232	0.24	(−0.06, 0.55)	0.32	(0.01, 0.64)	0.33	(−0.03, 0.70)
Per pregnancy		0.02		0.04		0.04	
		p-trend = 0.65		*p*-trend = 0.29		*p*-trend = 0.42	
Cumulative total breastfeeding duration (mo.)							
0 – <1	171	REF	--	REF	--	REF	--
1 – 6	90	0.24	(−0.10, 0.58)	0.24	(−0.10, 0.59)	0.22	(−0.14, 0.59)
7 – 12	120	0.07	(−0.24, 0.38)	0.07	(−0.25, 0.39)	0.05	(−0.30, 0.40)
13 – 18	126	0.35	(0.04, 0.66)	0.44	(0.12, 0.75)	0.42	(0.06, 0.77)
19 – 24	84	0.08	(−0.27, 0.43)	0.10	(−0.25, 0.46)	0.07	(−0.33, 0.48)
≥25	203	0.08	(−0.19, 0.36)	0.17	(−0.11, 0.46)	0.14	(−0.21, 0.49)
Per month		<.0.0001		0.002		0.0004	
		p-trend = 0.99		*p*-trend = 0.48		*p*-trend = 0.91	
Cumulative exclusive breastfeeding duration (mo.)							
0 – <1	313	REF	--	REF	--	REF	--
1 – 6	153	−0.03	(−0.29, 0.23)	−0.07	(−0.33, 0.20)	−0.08	(−0.35, 0.19)
7 – 12	186	0.06	(−0.18, 0.30)	0.08	(−0.17, 0.32)	0.06	(−0.20, 0.32)
13 – 18	99	0.02	(−0.28, 0.32)	0.09	(−0.22, 0.40)	0.06	(−0.27, 0.40)
≥19	34	0.28	(−0.19, 0.75)	0.40	(−0.09, 0.88)	0.35	(−0.18, 0.88)
Per month		0.01		0.01		0.01	
		p-trend = 0.41		*p*-trend = 0.16		*p*-trend = 0.31	

MV1: adjusted for age at blood collection, assay (fasting status [dichotomous], time of blood collection [4 pm, 3 pm, 1–2pm, 5pm–12am], season of blood collection [winter, spring, fall, summer], luteal day [0–5, 6–9, 10–14, ≥15 days])), and AMH level at first blood collection

MV2: MV1 + further adjusted for pack-years of smoking (continuous), smoking status (never, current, former), BMI (continuous), exogenous hormone use (dichotomous), duration of oral contraceptive use (never, 1–23, 24–71, 72–119, ≥120 months), years until cycle became regular (never, <1, 1 – 2, 3 – 4, ≥5.0 years), infertility history (dichotomous), age at menarche (≤11, 12, ≥13 years), and alcohol intake (never, ≤6 drinks per week, 1 drink per day, ≥2 drinks per day)

MV3: MV2 + further adjusted for other reproductive factor: cumulative total breastfeeding duration (continuous) or parity (continuous)

Table 4.

Relation of mean age at menopause with parity and breastfeeding, accounting for AMH levels, Nurses' Health Study 2.

Reproductive Factor	No. of Participants (n=1389)	MV1	MV1 + Additionally adjusted for AMH
		LS MEANS (SE)	LS MEANS (SE)
Parity			
0	270	49.7 (0.3)	49.9 (0.2)
1	222	50.3 (0.3)	50.2 (0.2)
2	505	50.1 (0.2)	50.1 (0.1)
≥3	392	50.1 (0.2)	50.2 (0.1)
Per pregnancy		0.03	0.04
		p-trend = 0.76	*p*-trend = 0.61
Cumulative total breastfeeding duration (mo.)			
0 – <1	379	49.6 (0.3)	50.0 (0.2)
1 – 6	152	49.8 (0.3)	50.1 (0.2)
7 – 12	207	50.0 (0.3)	50.1 (0.2)
13 – 18	198	50.1 (0.3)	50.0 (0.2)
19 – 24	132	50.1 (0.2)	50.2 (0.2)
≥25	321	50.8 (0.2)	50.2 (0.2)
Per month		0.03	0.01
		p-trend = 0.002	*p*-trend = 0.25
Cumulative exclusive breastfeeding duration (mo.)			
0 – <1	622	49.9 (0.2)	50.1 (0.1)
1 – 6	245	49.8 (0.3)	49.8 (0.2)
7 – 12	296	50.2 (0.2)	50.1 (0.1)
13 – 18	145	50.7 (0.3)	50.2 (0.3)
≥19	64	50.6 (0.5)	50.2 (0.1)
Per month		0.05	0.01
		p-trend = 0.01	*p*-trend = 0.53

MV1: adjusted for age at first blood collection, pack-years of smoking (continuous), smoking status (never, current, former), BMI (continuous), exogenous hormone use (dichotomous), duration of oral contraceptive use (never, 1–23, 24–71, 72–119, ≥120 months), years until cycle became regular (never, <1, 1 – 2, 3 – 4, ≥5 years), infertility history (dichotomous), age at menarche (≤11, 12, ≥13 years), and alcohol intake (never, ≤6 drinks per week, 1 drink per day, ≥2 drinks per day), and other reproductive factor: cumulative total breastfeeding duration (continuous) or parity (continuous) (n=1619)

MV2: MV1 + further adjusted for AMH levels at first blood collection