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## Associations of reproductive and breastfeeding history with anti-Müllerian hormone concentrations among reproductive aged African-American women

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### Abstract

**Research Question:** Are gravidity, parity, and history of breastfeeding associated with anti-Müllerian hormone concentrations among reproductive-aged women?

**Design:** This study included baseline data from Study of the Environment, Lifestyle and Fibroids, a five-year longitudinal study of African-American women. Within this community cohort, data from 1,392 women aged 25-35 years were analyzed. The primary outcome was serum anti-Müllerian hormone concentration measured using the Ansh Labs picoAMH assay, an enzyme linked immunosorbent assay. We used multivariable linear regression models to estimate mean differences in anti-Müllerian hormone concentrations ( $\beta$ ) and 95% confidence intervals

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(CI) by self-reported gravidity, parity, and breastfeeding history, with adjustment for potential confounders.

**Results:** Of the 1,392 participants, 1,063 were previously gravid (76.4%). Of the previously gravid participants, 891 (83.8%) were parous, 564 of whom had breastfed. In multivariable-adjusted regression analyses, there was no appreciable difference in anti-Müllerian hormone concentrations between nulligravid and participants with history of gravidity ( $\beta = -0.025$ , 95% CI [-0.145, 0.094]). Among previously gravid participants, there was little difference in anti-Müllerian hormone concentrations between parous and nulliparous participants ( $\beta = 0.085$ , 95% CI [-0.062, 0.232]). There was also little association with anti-Müllerian hormone concentrations according to history of breastfeeding (ever vs. never:  $\beta = 0.009$ , 95% CI [-0.093, 0.121]) or duration of breastfeeding (per 1-month increase):  $\beta = -0.002$ , 95% CI [-0.010, 0.006]).

**Conclusions:** Gravidity, parity, and history of breastfeeding were not meaningfully associated with anti-Müllerian hormone concentrations in this large sample of the Study of the Environment, Lifestyle and Fibroids cohort.

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## Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein that is a member of the transforming growth factor  $\beta$  family and is produced by granulosa cells of preantral and antral follicles in the ovary (Dewailly et al. 2014). Functionally, AMH is an important regulator of folliculogenesis through its inhibition of recruitment of primordial follicles (Visser and Themmen 2005). Measurement of AMH concentration in the serum is proportional to the quantity of developing ovarian follicles, and serum AMH concentration is clinically used to assess ovarian reserve (Dewailly et al. 2014). Although serum AMH concentration does not correlate with an individual's ability to conceive without medical assistance, it shows high specificity as a screening test for poor ovarian reserve (Streuli et al. 2014, Practice Committee of the American Society for Reproductive 2015, Steiner et al. 2017, Lin et al. 2021, Harris et al. 2022). AMH concentration also predicts ovarian response to gonadotropin stimulation during in-vitro fertilization (IVF) treatment (Practice Committee of the American Society for Reproductive 2015).

AMH can be utilized as a marker of ovarian aging; as the ovarian follicular pool declines with age, circulating AMH also declines (Broekmans et al. 2009). Accordingly, timing of menopause has been found to correlate with AMH concentrations, independent of age (van Disseldorp et al. 2008, Freeman et al. 2012, Dolleman et al. 2013). Although there is generally a progressive reduction in AMH concentrations with age, some individuals have lower or higher AMH concentrations than would be expected for their age (La Marca et al. 2012, Practice Committee of the American Society for Reproductive 2015). This indicates that other factors also influence AMH; such factors may include endometriosis, polycystic ovary syndrome, body mass index and race/ethnicity (Oh et al. 2019). Several prior studies have demonstrated that parity and history of breastfeeding are related to the timing of menopause (Ortega-Ceballos et al. 2006, Gold 2011, Li et al. 2012, Langton et al. 2020). It is hypothesized that through inhibition of ovulation, pregnancy and breastfeeding may delay the loss of the ovarian follicular pool (Langton et al. 2020). This theory has led to questions

of the extent to which AMH concentrations are influenced by a history of pregnancy and breastfeeding.

Numerous studies have examined factors that impact AMH beyond age and some have evaluated the association between reproductive history and AMH concentrations (Bragg et al. 2012, La Marca et al. 2012, Moini et al. 2016, Jung et al. 2017). However, the association between an individual's pregnancy history and AMH concentrations has been inconsistent, with some studies showing higher AMH concentrations among parous women, after accounting for age, while others have found no association (Nardo et al. 2007, Bragg et al. 2012, La Marca et al. 2012, Dólleman et al. 2013, Moini et al. 2016, Catteau-Jonard et al. 2017, Jung et al. 2017). The association between a history of breastfeeding and AMH concentrations has also not been well established, with one prior study finding higher AMH concentrations in women who breastfed and another finding no association between AMH and breastfeeding history (Whitworth et al. 2015, Grimes et al. 2022). Therefore, the objective of this study was to evaluate the association of pregnancy and breastfeeding history and AMH concentrations in a large cohort of reproductive-aged women.

## Materials and Methods

### Study Participants

The Study of Environment, Lifestyle and Fibroids (SELF) is a prospective cohort study; previous publications describe the study design, methods, enrollment and cohort population (Baird et al. 2015, Bernardi et al. 2017, Bernardi et al. 2022). In brief, 1,693 reproductive-age residents of the Detroit, Michigan area, ages 23-35, who affirmed being "African-American or Black" enrolled between November 2010 and December 2012. Individuals with a history of uterine fibroid diagnosis, hysterectomy, or medical treatment for cancer or an autoimmune disorder were excluded from participation. For participants pregnant at recruitment, enrollment was delayed until at least 3 months post-pregnancy. Gender identity information was not collected in this cohort. The institutional review boards at participating institutions approved the study, and all participants provided written informed consent. We restricted our analyses to the 1,392 participants aged 25-35 years because AMH concentrations did not peak until age 25 in the SELF cohort (Marsh et al. 2016). Other studies have also demonstrated that AMH concentration continues to rise at the start of an individual's third decade of life (La Marca et al. 2010, Tehrani et al. 2014, Cui et al. 2016). This study is limited to analysis of data obtained at the time of the enrollment visit, which also included assessment of participant height and weight, a non-fasting venous blood draw, and completion of interviews and self-administered questionnaires.

### Evaluation of AMH

The assessment of AMH concentrations in this cohort is previously described (Bernardi et al. 2022). The enrollment visit was at a variable timepoint within participants' menstrual cycles. Variation in AMH concentration across the menstrual cycle is low and timed measures are not required for clinical practice (Kissell et al. 2014). Serum from enrollment was stored at  $-80^{\circ}\text{C}$  until thawed once and re-frozen for processing for long term storage. Six to eight years after the serum was initially obtained, frozen samples were shipped to

Ansh labs (Webster, Texas, USA), where AMH assays were performed using the picoAMH assay, an enzyme linked immunosorbent assay (ELISA). The same reagent lot was used for all samples. AMH values are presented in concentration of ng/mL with the lower limit of detection of the test being 1.3 pg/mL. Intra-assay and inter-assay coefficients of variation were <5%. There were 1,392 participants aged 25-35 with serum AMH concentrations available for analysis. There were three participants with AMH values below the lower limit of detection, and these participants were assigned a value of 0.001 ng/mL using an established formula (Hornung and Reed 1990).

### Assessment of Reproductive History and Covariates

In order to determine gravidity and parity history, self-reported data on the number and timing of all pre-enrollment pregnancies and deliveries were obtained via computer-assisted telephone interview. Details on whether a participant breastfed, and duration of breastfeeding were also determined in this manner. A participant was classified as “gravid” if they reported having at least one previous pregnancy and “parous” if they reported having at least one previous delivery, including stillbirths.

Body mass index (BMI) was calculated from height and weight measured at the enrollment visit. Participants reported history of polycystic ovary syndrome (PCOS), “seeking care for difficulty conceiving”, abnormal menstrual bleeding, and thyroid conditions. Current use of hormonal contraception including oral contraceptive pills, vaginal ring, hormonal shot, hormonal patch, hormonal implant, and hormonal intrauterine device was also assessed. Menstrual cycle length over the previous 12 months, educational level, annual household income, and smoking status were also self-reported.

### Statistical Analysis

Primary analyses assessed AMH concentrations comparing gravid with nulligravid participants, parous with nulliparous participants, and participants who had breastfed with those who had not breastfed.

The distribution of AMH, the explanatory variables of interest, and relevant participant characteristics were described by means (with standard deviations [SD]) or medians (with interquartile ranges [IQR]) for continuous variables and proportions for categorical variables. Given that AMH concentrations were not normally distributed, they were log-transformed for analysis in linear regression models. As age has been consistently found to be associated with AMH concentrations, including in a previous analysis of this cohort, age-adjusted linear regression was performed to evaluate the association between the variables of interest and AMH.(Marsh et al. 2016) Both age and quadratic age were included in the model to allow for a nonlinear association between AMH and age. Additional prior analyses of this cohort have found a significant association between AMH and BMI, current hormonal contraceptive use, history of a thyroid condition, self-reported history of abnormal menstrual bleeding and “seeking care for difficulty conceiving ”(Marsh et al. 2016). Estimates of mean differences in AMH concentrations ( $\beta$ ), 95% confidence intervals (CI), and *P* values were obtained from linear regression analyses. We also performed sensitivity analyses excluding women with a self-reported diagnosis of PCOS (n=45) and an

outlier participant with an AMH concentration of 55.7 ng/mL. Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC) was used to perform all analyses with  $P < 0.05$  representing statistical significance.

## Results

### Baseline Characteristics

The mean age of included participants was  $30.1 \pm 2.9$  years and the median AMH concentration was 4.0 ng/mL (IQR 2.2, 6.6). The majority of the cohort examined was gravid (76.4%). The mean and median unadjusted AMH concentrations in gravid and nulligravid participants are shown in Table 1. Demographic characteristics, and medical and reproductive history by gravidity are also described in Table 1. The unadjusted AMH concentrations by gravidity are shown in Figure 1. Of the gravid individuals, 891 were parous (83.8%), and 564 participants had a history of breastfeeding (40.5% of entire cohort, 63.3% of parous individuals). Pregnancy and breastfeeding details for gravid participants are shown in Table 2.

### Association of gravidity, parity and AMH

The unadjusted median AMH concentration among gravid participants was lower at baseline (3.8 ng/mL, IQR [2.2, 6.1 ng/mL]) compared to nulligravid participants (5.0 ng/mL, IQR [2.4, 7.7 ng/mL];  $p < 0.001$ ) (Table 1). However, as shown in Table 3, in age-adjusted and multivariable-adjusted analyses, there was no appreciable association between gravidity and log-transformed AMH concentration (age-adjusted  $\beta = -0.064$ , 95% CI [-0.183, 0.054]; multivariable-adjusted  $\beta = -0.025$ , 95% CI [-0.145, 0.094]). When the number of prior pregnancies was examined continuously among gravid participants, there was little association with log-transformed AMH concentration (age-adjusted  $\beta = -0.008$ , 95% CI [-0.034, 0.019]; multivariable-adjusted  $\beta = 0.004$ , 95% CI [-0.031, 0.022]; Table 3). Results were similarly null when we compared parous participants with those who were gravid but nulliparous (age-adjusted  $\beta = 0.015$ , 95% CI [-0.131, 0.161], multivariable-adjusted  $\beta = 0.085$ , 95% CI [-0.062, 0.232]), or when parity was examined continuously among parous participants (age-adjusted  $\beta = -0.035$ , 95% CI [-0.080, 0.010]); multivariable-adjusted  $\beta = -0.030$ , 95% CI [-0.075, 0.015]; Table 3).

### Association of breastfeeding and AMH

The unadjusted median AMH concentration among the 564 participants who had a history of breastfeeding was 3.8 ng/mL (IQR 2.1-6.1 ng/mL) compared to all those who had never breastfed, regardless of parity (4.1 ng/mL, IQR 2.2-7.1 ng/mL;  $p = 0.134$ ), but no significant differences in log-transformed AMH concentrations were seen in either age-adjusted or multivariable-adjusted analyses (age-adjusted  $\beta = -0.023$ , 95% CI [-0.125, 0.078]; multivariable-adjusted  $\beta = 0.009$ , 95% CI [-0.093, 0.111]; Table 4). Results were similar when nulligravid women were excluded, (age-adjusted  $\beta = 0.002$ , 95% CI [-0.105, 0.109], multivariable-adjusted  $\beta = 0.014$ , 95% CI [-0.093, 0.121]; Table 4), and when only parous women were examined (age-adjusted  $\beta = -0.002$ , 95% CI [-0.121, 0.117]; multivariable-adjusted  $\beta = -0.022$ , 95% CI [-0.142, 0.098]; Table 4). The mean duration of breastfeeding in our cohort was 6 months (IQR [2, 12 months]). Cumulative duration of

breastfeeding, among those who breastfed, showed no association with AMH concentration (age-adjusted  $\beta = -0.001$ , 95% CI  $[-0.009, 0.007]$ ; multivariable-adjusted  $\beta = -0.002$ , 95% CI  $[-0.010, 0.006]$ ; Table 4).

Exclusion of participants with PCOS or exclusion of a single participant with an AMH concentration  $>50$  ng/mL did not alter interpretation of our results.

## Discussion

In this large study of reproductive-aged African-American women, we did not find any clinically meaningful differences in AMH concentrations by gravidity or parity status. Furthermore, there was no association between history of breastfeeding or cumulative months of breastfeeding and AMH concentrations. These findings suggest that gravidity, parity and breastfeeding are not predictors of AMH concentration among female participants aged 25-35 years.

Existing studies that have evaluated the association between parity and AMH have yielded conflicting results. Studies finding an increase in AMH with increasing parity were among younger populations or were not fully controlled for age (Bragg et al. 2012, Dólleman et al. 2013, Moini et al. 2016). Many studies, like ours, have found no association between parity and AMH concentrations. La Marca et al. performed a cross-sectional investigation of 277 healthy women in Italy aged 18-50 years and reported that AMH concentrations were independent of parity (La Marca et al. 2010). An international cross-sectional study of 671 premenopausal women mostly in their late thirties to early forties also noted no association between AMH concentrations and parity (Jung et al. 2017). In a study designed to establish age-specific reference values for AMH level, AMH levels were found to be independent of parity in a cohort of 416 Italian healthy eumenorrheic women with a median age of 34 years (La Marca et al. 2012). Among a cohort of 217 oocyte donors in France with at least one live birth and a median age of 32 years, there was no significant correlation between AMH concentrations and parity (Catteau-Jonard et al. 2017). When 136 women with infertility in the United Kingdom who were undergoing IVF were evaluated, there was also no significant association found between reproductive history and baseline AMH concentrations (Nardo et al. 2007).

The association between breastfeeding and AMH concentrations has not been well explored. Grimes et al. evaluated associations between AMH and reproductive history in a subset of premenopausal participants ages 32 to 46 years in the Nurses' Health Study II and found that mean log AMH levels were 39 percent higher in women reporting 25 months or more of total breastfeeding compared to less than 1 month of breastfeeding ( $P$  for trend = 0.009) (Grimes et al. 2022). We did not find an association between breastfeeding history or duration and AMH concentrations, however the median total cumulative breastfeeding duration in our cohort was 6 months while the largest group of women who breastfed in the Nurses' Health Study did so for 25 months or more (Grimes et al. 2022). While it is possible that a longer total breastfeeding duration is associated with AMH concentrations, this was rare in our cohort. Additionally, a study of 420 South African women from 2010-2011 found no association between log-transformed AMH concentration and total breastfeeding duration



when comparing total breastfeeding 0-17 months, 18-27 months and greater than 28 months (Whitworth et al. 2015). Our findings add to this limited data suggesting no association between breastfeeding history or duration and AMH concentrations and specifically evaluate this association in African-American women.

While there is speculation that long periods of anovulation, associated with prolonged breastfeeding and multiple gestational periods, would delay depletion of the ovarian follicular pool and thereby be associated with higher AMH concentrations, evidence for this is lacking (Dólleman et al. 2013, Jung et al. 2017, Langton et al. 2020). Our unadjusted comparison actually showed a lower median AMH concentration among gravid compared with nulligravid women, counter to the hypothesized comparison. However, when just age-adjusting or additionally adjusting for important covariates including BMI, current hormonal contraceptive use, history of a thyroid condition, self-reported history of abnormal menstrual bleeding, and “seeking care for difficulty conceiving”, the associations were null.

Since many women in our cohort had delivered or completed breastfeeding years prior, we were not able to evaluate the short-term impact of pregnancy and breastfeeding on AMH concentrations. A short-term decline in AMH concentration following a pregnancy loss or birth may be important for women with infertility who may be having repeat testing to guide decision-making surrounding family building or considering pursuing IVF. However, our findings should provide reassurance that there is no evidence of a long-term reduction in ovarian reserve following pregnancy, delivery, or breastfeeding.

Future study of reproductive and breastfeeding history and ovarian reserve could benefit from a prospective design to better understand the trajectory of AMH concentrations over an individual’s reproductive lifespan. This would additionally allow for evaluation of any short-term impact of pregnancy, delivery or breastfeeding on an individual’s ovarian reserve. Research on ovarian reserve will also benefit from inclusion of participants of diverse racial and ethnic backgrounds.

There are some limitations of our data to consider. Reproductive history and breastfeeding were self-reported and may have been measured with error. However, this error was likely to be random, as participants were unaware of the AMH concentrations and prior studies have demonstrated good maternal recall of breastfeeding duration (Li et al. 2005, Natland et al. 2012). Due to the number of participants, we analyzed gravidity and parity categorically and continuously. Additionally, given the age distribution of our cohort, many women may not have yet completed childbearing or the full duration of breastfeeding. Further, participants’ AMH concentrations were not analyzed longitudinally, precluding the ability to assess AMH levels during and after pregnancy and breastfeeding. While only a single AMH value was considered in this analysis, clinicians typically make decisions and guide patient care on the basis of a single measurement. Finally, the serum samples had been previously thawed and refrozen and were stored for several years prior to AMH concentration being analyzed for this study. However, we expect given their storage at –80 degrees that concentrations should remain stable over time, and prior work has shown stability of AMH in serum during repeated thawing and freezing cycles (Morse et al. 2016, Vrzáková et al. 2023).



There are several strengths of this study. Participants were not selected on the basis of menstrual cycle or fertility characteristics, providing broader generalizability. It provides data on a large community cohort of reproductive-aged African-American women. The exclusion of women who did not identify as “African-American or Black” from this cohort is not expected to limit generalizability, and the trajectory of declining AMH with age is thought to be concordant across ethnicities (Kotlyar and Seifer 2021). The mean age of participants was 30.1 years (range 25-35 years), which makes the findings relevant at an important time point of the reproductive lifespan. Further, the SELF cohort has been well characterized, enhancing the ability to identify and control for several potential confounders. Additionally, 564 participants had breastfed which allowed for a well-powered analysis of the association between a history of breastfeeding and AMH concentrations.

Our study demonstrates that gravidity and parity were not associated with AMH concentrations in reproductive-aged African-American women. Further, history of breastfeeding and cumulative duration of breastfeeding were not associated with AMH concentrations.

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## Biography



Dr. Komorowski is a fellow in Reproductive Endocrinology & Infertility at Northwestern University. She completed medical school and OB/GYN residency at Washington University in St. Louis. Her research interests include ovarian reserve and social determinants of health in reproductive medicine.

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### Highlights

- Gravidity and parity were not associated with AMH levels in reproductive-aged women
- History of breastfeeding was not associated with AMH concentrations
- There is no long-term reduction in ovarian reserve with prior pregnancy or delivery

**Key Message:**

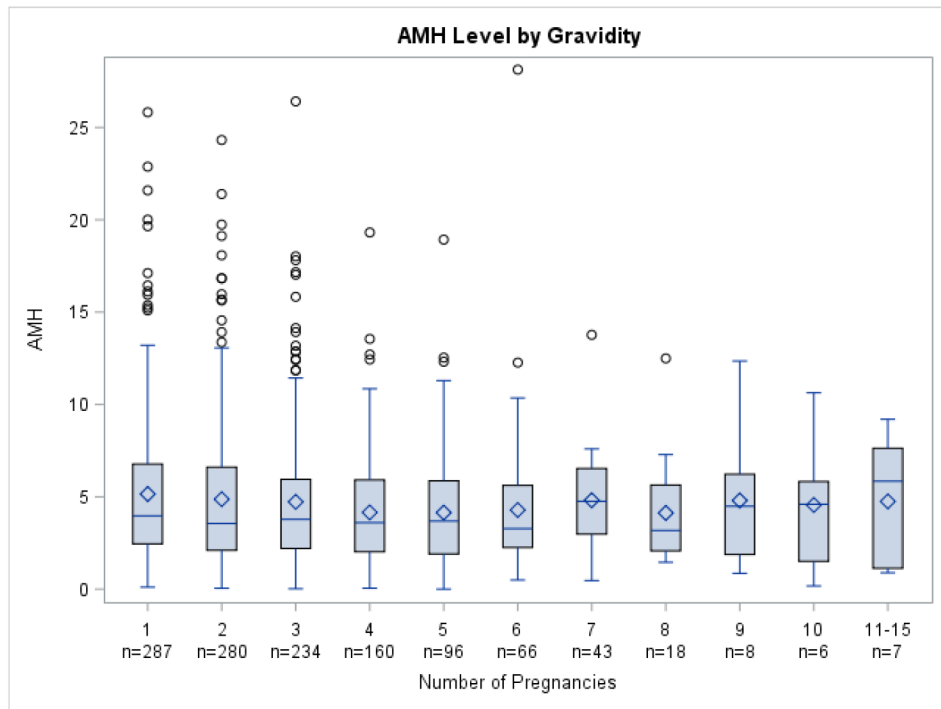
Gravidity, parity, and history of breastfeeding were not meaningfully associated with anti-Müllerian hormone concentrations in this large, well-characterized community cohort of reproductive-aged women. These findings provide reassurance that there is no long-term reduction in ovarian reserve noted in women with a history of pregnancy, delivery, or breastfeeding.

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**Figure 1:** Unadjusted anti-Müllerian hormone (AMH) concentration by number of pregnancies (no statistically significant difference). \*Excludes outlier participant with AMH 55.7 ng/mL



**Table 1:**

Baseline characteristics of gravid and nulligravid study participants (n=1392)

	Gravid	
	No (n=329)	Yes (n=1063)
Age at enrollment, y (mean $\pm$ SD; range) <sup>a</sup>	29.3 $\pm$ 2.9 (25-35.0)	30.4 $\pm$ 2.9 (25-35.8)
AMH, ng/mL (mean $\pm$ SD; range)	5.7 $\pm$ 4.4 (0.001-27.9)	4.7 $\pm$ 4.0 (0.001-55.7)
AMH, ng/mL (median, IQR)	5.0 (2.4, 7.7)	3.8 (2.2, 6.1)
BMI, kg/m <sup>2</sup> (mean $\pm$ SD)	34.9 $\pm$ 10.7	33.7 $\pm$ 9.1
History of PCOS (%)	6.4%	2.3%
History of abnormal menstrual bleeding (%)	18.8%	10.0%
History of a thyroid condition (%)	3.3%	3.3%
Hormonal contraception use within the past 4 weeks (%)	24.6%	31.8%
Menstrual cycle length >35 days or irregular (%)	5.5%	5.0%
Education Level		
High school/GED or less	13.4%	23.6%
Some college/Associate's/Technical	38.9%	53.3%
Bachelor's/Master's/PhD	47.7%	23.0%
Decline to answer	0%	0.1%
Annual household income		
<\$20,000	35.9%	46.3%
\$20,000-50,000	41.6%	35.8%
>\$50,000	21.9%	17.2%
Declined to answer or Unsure	0.6%	0.7%
Smoking status		
Never	76.9%	72.1%
Former	6.4%	8.2%
Current (<10 cigarettes/day)	10.6%	14.2%
Current ( 10 cigarettes/day)	6.1%	5.6%

<sup>a</sup>Only individuals under 35 were recruited, but some had turned 35 by the time that all baseline activities and enrollment were completed.

\* Abbreviations: AMH = anti-Müllerian hormone, BMI = body mass index, GED = General Education Development, PhD = Doctor of Philosophy

**Table 2:**

Pregnancy and breastfeeding details in gravid participants (n=1063)

	<b>Gravid but nulliparous (n=172)</b>	<b>Parous without history of breastfeeding (n=327)</b>	<b>Parous with history of breastfeeding (n=564)</b>
Number of pregnancies (mean $\pm$ SD; range)	1.7 $\pm$ 1.0 (1-7)	3.6 $\pm$ 2.2 (1-15)	3.5 $\pm$ 2.1 (1-13)
Years since last pregnancy (mean $\pm$ SD; range)	6.4 $\pm$ 4.5 (0-18.0)	5.1 $\pm$ 4.0 (0.1-18.4)	3.9 $\pm$ 3.2 (0.1-17.4)
Number of deliveries (mean $\pm$ SD; range)	--	2.2 $\pm$ 1.4 (1-11)	2.1 $\pm$ 1.2 (1-8)
Years since last delivery (mean $\pm$ SD; range)	--	6.5 $\pm$ 4.2 (0.1-18.4)	4.8 $\pm$ 3.5 (0.2-17.4)
Cumulative months of breastfeeding all children (mean $\pm$ SD; range)			8.7 $\pm$ 9.6 (0.3-60.0)

\* Abbreviations: SD = standard deviation

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**Table 3:**

The association between reproductive history and anti-Müllerian hormone (AMH) concentrations

Predictor	N	Age-adjusted model <sup>a</sup>	Multivariable model <sup>b</sup>
		$\beta^c$ (95% CI)	$\beta^c$ (95% CI)
<b>Gravid</b>			
No	329	Reference	Reference
Yes	1063	-0.064 (-0.183, 0.054)	-0.025 (-0.145, 0.094)
<b>Gravidity continuous (per 1-pregnancy increase)<sup>d</sup></b>	1063	-0.008 (-0.034, 0.019)	0.004 (-0.031, 0.022)
<b>Parous<sup>e</sup></b>			
No	172	Reference	Reference
Yes	891	0.015 (-0.131, 0.161)	0.085 (-0.062, 0.232)
<b>Parity (per 1-birth increase)<sup>f</sup></b>	891	-0.035 (-0.080, 0.010)	-0.030 (-0.075, 0.015)

<sup>a</sup>The model included adjustment for age and (age)<sup>2</sup>.

<sup>b</sup>The multivariable model included adjustment for age, (age)<sup>2</sup>, BMI, current use of any hormonal contraceptive, abnormal menstrual bleeding, history of any thyroid condition, "seeking care for difficulty conceiving", and PCOS.

<sup>c</sup> $\beta$ -coefficient represents difference in log-transformed AMH concentration between comparison or reference category or the change in log-transformed AMH concentration for each one unit increase in the predictor being analyzed.

<sup>d</sup>Only gravid participants examined.

<sup>e</sup>Only gravid participants examined. The reference group is participants who were gravid but nulliparous.

<sup>f</sup>Only parous participants examined.

**Table 4:**

The association between breastfeeding and anti-Müllerian hormone (AMH) concentrations

Predictor	N	Age-adjusted model <sup>a</sup>	Multivariable model <sup>b</sup>
		$\beta^c$ (95% CI)	$\beta^c$ (95% CI)
<b>History of breastfeeding among all participants <sup>d</sup></b>			
No	828	Reference	Reference
Yes	564	-0.023 (-0.125, 0.078)	0.009 (-0.093, 0.111)
<b>History of breastfeeding among gravid participants <sup>e</sup></b>			
No	499	Reference	Reference
Yes	564	0.002 (-0.105, 0.109)	0.014 (-0.093, 0.121)
<b>History of breastfeeding among parous participants <sup>f</sup></b>			
No	327	Reference	Reference
Yes	564	-0.002 (-0.121, 0.117)	-0.022 (-0.142, 0.098)
<b>Cumulative months of breastfeeding (per 1 month increase) <sup>g</sup></b>			
	564	-0.001 (-0.009, 0.007)	-0.002 (-0.010, 0.006)

<sup>a</sup>The model included adjustment for age and (age)<sup>2</sup>

<sup>b</sup>The multivariable model included adjustment for age, (age)<sup>2</sup>, BMI, current use of any hormonal contraceptive, abnormal menstrual bleeding, history of any thyroid condition, “seeking care for difficulty conceiving”, and PCOS.

<sup>c</sup> $\beta$ -coefficient represents difference in log-transformed AMH concentration between those who breastfed and those who did not or change in log-transformed AMH concentration for each additional cumulative month of breastfeeding

<sup>d</sup>All participants examined. The reference group is participants who never breastfed regardless of parity.

<sup>e</sup>Restricted to gravid participants. The reference group is gravid participants who never breastfed regardless of parity.

<sup>f</sup>Restricted to parous participants. The reference group is parous participants who never breastfed.

<sup>g</sup>Months of breastfeeding evaluated continuously among participants who had breastfed.

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