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Early-life farm exposure and ovarian reserve in a U.S. cohort of women

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Abstract

Background: In a previous exploratory study, we reported lower concentrations of the ovarian reserve biomarker anti-Müllerian hormone (AMH) in adulthood with prenatal farm exposure. We now examine this association as well as childhood farm exposure using enrollment data from the Sister Study, a large U.S. cohort of women.

Methods: We collected prenatal and childhood farm exposure data by questionnaire and telephone interview. However, serum AMH data were available only for a nested subset: premenopausal women ages 35–54 subsequently diagnosed with breast cancer (n=418 cases) and their matched controls (n=866). To avoid potential bias from restricting analyses to only premenopausal controls, we leveraged the available cohort data. We used data from both premenopausal cases and controls as well as postmenopausal women ages 35–54 (n=3,526) (all presumed to have undetectable AMH concentrations) and applied weights to produce a sample representative of the cohort ages 35–54 (n=17,799). The high proportion of undetectable AMH concentrations (41%) was addressed using reverse-scale Cox regression. An adjusted hazard ratio (HR) <1.0 indicates that exposed individuals had lower AMH concentrations than unexposed individuals.

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Data and computing code: Researchers interested in replicating the results can request access to study data and coding from the National Institute of Environmental Health Sciences Sister Study Tracking and Review System (STaRS) website at https://www.sisterstudystars.org.

Results: Prenatal exposure to maternal residence or work on a farm was associated with lower AMH concentrations (HR 0.66, 95% CI: 0.48–0.90). Associations between childhood farm residence exposures and AMH were null or weak, except childhood contact with pesticide-treated livestock or buildings (HR 0.69, 95% CI: 0.40–1.2).

Conclusions: Replication of the prenatal farm exposure and lower adult AMH association raises concern that aspects of prenatal farm exposure may result in reduced adult ovarian reserve.

Keywords

early life; farm; anti-Müllerian hormone; ovarian reserve

Introduction

Natural menopause occurs after the number of oocytes in the ovaries, the ovarian reserve, falls below a critical threshold.[1,2] The ovarian reserve consists of a pool of primordial follicles (oocytes surrounded by a single layer of granulosa cells) established *in utero.*[3] The longevity of ovarian function is determined by the size of the prenatally established ovarian reserve, rate of subsequent primordial follicle activation and atresia, and direct loss. The loss of primordial follicles occurs not only from the cyclic recruitment of ovarian follicles after puberty, but also from the continuous activation of primordial follicles and their atresia *in utero* and postnatally, both before and after puberty.[3,4] Early loss of primordial follicles is estimated to be substantial, with 50% of the prenatal ovarian reserve being lost before approximately age 16.[5] Thus, the intrauterine and childhood periods are susceptible developmental windows during which the ovarian reserve could be adversely affected by ovarian-toxic exposures.

In a prior exploratory study investigating a range of 32 early-life factors in relation to anti-Müllerian hormone (AMH) concentrations, we observed that prenatal exposure to the farm environment through maternal residence or work on a farm was associated with lower AMH concentrations in adulthood.[6] AMH is a biomarker of ovarian reserve and its concentration reflects the number of recruitable ovarian follicles, which is proportional to the size of the primordial follicle pool.[7] We hypothesized that the lower AMH concentrations observed with prenatal farm exposure may be due to pesticide exposure,[6] based on supporting evidence from human[8,9] and *in vivo* and *in vitro* laboratory studies.[10–14] However, replication of that finding is warranted: multiple testing could have given rise to a chance association, few participants reported prenatal farm exposure (17 exposed among 1500 participants), and no association was observed between any childhood farm residence and AMH. Therefore, the purpose of the present study was to further evaluate the association between prenatal and childhood exposure to the farm environment and AMH in women ages 35–54 years using data from a large U.S. epidemiologic cohort that collected detailed data on childhood farm residence.

Methods

Data source

We used enrollment data from the Sister Study, an ongoing prospective cohort study of genetic and environmental risk factors for breast cancer incidence that enrolled 50,884 women ages 35–74 years residing in the United States, including Puerto Rico in years 2003–2009. Details regarding recruitment, enrollment, and data collection have been previously described.[15] At enrollment, participants completed a computer-assisted telephone interview and self-administered questionnaires, which collected information on demographics, lifestyle factors, and medical and reproductive history. Participants also were visited by study staff in their homes during which anthropometry data and biologic specimens were collected; 99.1% of participants provided a blood sample at enrollment.[15] Each participant provided informed consent prior to enrollment. The conduct of the Sister Study was approved by the Institutional Review Boards at the National Institute of Environmental Health Sciences, NIH, and the Copernicus Group.

Nested within the Sister Study cohort, a matched case–control study was conducted to investigate serum AMH concentrations in relation to breast cancer risk.[16] The nested case–control study included 458 incident breast cancer cases diagnosed between enrollment and December 31, 2012, and 916 controls who were matched to cases on age and year of study enrollment and who were free of breast cancer at the time of their matched case's diagnosis. At enrollment, women in the nested sample were ages 35–54 years and premenopausal, based on having at least one ovary and reporting one or more menstrual cycles in the prior 12-month period. Women ages 35–54 years currently using hormonal contraception or replacement therapy, with a history of endometrial ablation that stopped menses, or with a history of hysterectomy without bilateral oophorectomy were also eligible and categorized as premenopausal. In addition, to be eligible, participants were required to have an archived serum sample of sufficient volume from the enrollment visit; of the identified cases, 99% (n=458) had sufficient archived serum samples.[16]

Measurement of AMH

Serum samples from blood collected at enrollment for the 458 nested cases and 916 matched controls were quantified for AMH by the Reproductive Endocrinology Laboratory at the University of Southern California Keck School of Medicine using the Ultrasensitive AMH enzyme-linked immunosorbent assay (ELISA) (Ansh Labs, Webster, TX).[16] Samples with AMH concentrations below the limit of detection (LOD) of 0.07 ng/ml (167 cases and 368 controls) were re-analyzed using the more sensitive picoAMH ELISA assay with an LOD of 0.003 ng/ml. Final AMH values were below the LOD for 24% of cases (n=108) and 28% of controls (n=252).

Study population sampling and weighting

An analysis limited to just the premenopausal controls from the nested study could result in biased results due to the sampling of controls who were matched to breast cancer cases on age and year of study enrollment[16] and the exclusion of postmenopausal women. Selection bias from the exclusion of postmenopausal women in a study population of women ages

35–54 years could be substantial, if, as hypothesized, early-life farm exposures contribute to early ovarian aging.

We therefore took a different approach. We used data from both premenopausal nested cases and controls with AMH measurements, and data from Sister Study participants ages 35–54 years at enrollment who reported being postmenopausal (with presumed undetectable AMH concentrations as AMH concentrations become undetectable approximately 5 years prior to the onset of menopause[17]). We then constructed and applied sample weights to produce a study sample representative of the entire Sister Study cohort meeting the eligibility criteria for the present analyses.

Specifically, we started with Sister Study participants who were ages 35–54 years at enrollment, which included both premenopausal and postmenopausal women, subject to having an enrollment serum sample of sufficient volume, a requirement of the case–control study (n=20,614). We excluded study participants whose medical history precluded the assessment of the ovarian reserve at enrollment: women who reported the surgical removal of both ovaries prior to reaching natural menopause (n=2,289); women with a history of premenopausal hysterectomy whose status on the removal of both ovaries was unknown (n=9); and those who reported ever being told by a doctor or health professional that they had polycystic ovaries, PCOS, or Stein-Leventhal Syndrome (n=467) or who responded “don’t know” to this question (n=19). Polycystic ovarian syndrome (PCOS) is characterized by a greater number of growing follicles, including pre-antral and small antral follicles that produce AMH.[18–20] AMH concentrations may not be reflective of ovarian reserve among women with PCOS.[21]

We also excluded women who reported chemotherapy or radiation therapy before enrollment that induced the permanent cessation of menses (n=27) or whose status was unknown (n=2). Some chemotherapeutic agents are well-established ovarian toxicants[22] and would obscure our ability to investigate early-life farm exposures on ovarian reserve. In addition, we excluded two study participants whose age at enrollment blood draw was greater than 54 years. After these exclusions, the redefined study cohort consisted of 17,799 study participants (eFigure 1).

We used this redefined study cohort to construct sample weights for the premenopausal nested cases and controls and postmenopausal study participants. We assigned nested cases and postmenopausal women a weight of 1.0 because all such women under age 55 years at enrollment were sampled. We assigned nested controls a sampling weight that was the inverse of the probability of being selected as a matched control among the pool of non-case study participants ages 35–54 years who were premenopausal at enrollment (n=13,833). To estimate the probability of being selected, we used logistic regression and included the nested case–control study matching factors of exact age at enrollment and year of enrollment in the model, as well as a quadratic term for age and terms for interactions between each of the two age variables and year of enrollment.

After we assigned sample weights, we excluded participants with AMH concentrations at or above the 99th percentile by age (n=39), based on the assumption that those with

undiagnosed PCOS would have the highest AMH concentrations and the lack of consensus on a cutoff value for the diagnosis of PCOS.[23] We also excluded two participants with low-quality AMH data.[16,24] This resulted in weighted data from 4,810 study participants: 418 premenopausal nested cases (2% of weighted sample), 866 premenopausal nested controls (77% of weighted sample), and 3,526 postmenopausal women (20% of weighted sample) for the present analyses (eFigure 1). Details on the prevalence of hysterectomy without bilateral oophorectomy and endometrial ablation that stopped menses in premenopausal participants are provided in eAppendix 1.

Prenatal and childhood farm exposures

At enrollment, participants were mailed a family history questionnaire, along with a pre-paid phone card to contact the participant's mother or other relatives about early-life exposures.[25] The family history questionnaire included questions on maternal farm exposure, including whether the participant's mother lived on a farm or did farm work any of the time she was pregnant with the participant. The response options were "definitely," "probably," "probably not," "definitely not," and "I don't know". We considered those who responded "definitely" or "probably" as exposed and "probably not" or "definitely not" as unexposed. Those answering "I don't know" were considered missing. Using these data, we created a binary exposure variable characterizing maternal residence or work on a farm (yes, no).

Data on childhood farm exposures were collected by computer-assisted telephone interview. Participants reporting that they ever lived on a farm ≥12 months during their lifetime, or that their current residence, residence longest lived as an adult, or residence longest lived before age 14 was ever used as a farm or orchard while living there, were asked to additionally complete a residential farm exposure module. In this module, participants were asked whether they had lived on a farm for ≥12 months at any time from birth up to age 18 and the characteristics of the farm. A farm was defined as "where crops are grown or livestock is raised" and did not include small, personal gardens. Data collected on childhood farm characteristics included the types of crops and/or livestock raised; whether pesticides were ever used on crops; whether the participant personally mixed, helped others mix, or applied pesticides, or cleaned or helped clean the pesticide mixing or application equipment used; whether the participant was present in the fields at the same time or on the same day as when pesticides were being applied to crops; whether the participant had contact with livestock; and whether livestock animals or the buildings where livestock were kept were ever treated with pesticides. Using these data, we created a variable for childhood residence on a farm ≥12 months (yes, no). Given our interest in childhood farm pesticide exposure, we created four variables focusing on 1) pesticide use on crops, 2) personal handling of crop pesticides, 3) presence in field during pesticide application, and 4) contact with pesticide-treated livestock or buildings during childhood farm residence.

Statistical analyses

We conducted the statistical analyses using Stata version 15.1 (StataCorp, College Station, TX). We descriptively compared the distribution of characteristics between participants with and without prenatal exposure to the farm environment and childhood farm residence. We

also estimated the median AMH concentration and the interquartile range (IQR) for each participant characteristic.

To evaluate the association between early-life farm exposures and AMH concentrations, we estimated the hazard ratio (HR) and 95% confidence interval (CI) using a reverse-scale Cox-regression-based approach. This approach allowed us to handle the high proportion of AMH concentrations below the LOD (41%, weighted), compared to other approaches such as substitution and multiple imputation, which can produce bias when the proportion of missing is substantial.[26] Concentrations below the LOD can be viewed as left-censored observations. To accommodate the Cox proportional hazards model, we converted the left-censored AMH concentration data to right-censored data by selecting a fixed constant (16.0 ng/ml) that exceeded the maximum AMH value in the dataset (15.80 ng/ml) and subtracting each observed AMH value from this maximum value. This conversion provided AMH concentrations on the reverse-scale. Participants with AMH concentrations below the LOD (0.003 ng/ml) were considered right-censored at AMH concentrations of 15.997 ng/ml on the reverse-scale, whereas those with detectable concentrations were considered uncensored. In the reverse-scale Cox regression, AMH concentration is the outcome and the HR is the ratio of the hazard of having a given reverse-scale AMH concentration among all reverse-scale concentrations at least that high for exposed individuals compared to unexposed individuals. In the reverse-scale analysis, HR>1.0 indicates that exposed individuals have lower reverse-scale AMH concentrations (and thus higher AMH concentrations) than unexposed individuals. Similarly, HR<1.0 indicates that exposed individuals have higher reverse-scale AMH concentrations (and thus lower AMH concentrations) than unexposed individuals. In the present analyses, we hypothesized that early-life farm exposure would be associated with lower ovarian reserve, and hence lower AMH concentrations, in adulthood (i.e., HR<1.0).

We selected variables for adjustment *a priori* based on associations reported in the AMH literature. In all analyses, we adjusted for exact participant age at blood draw, its quadratic term, and participant education (≤high school/GED, some college/Associate or technical degree, Bachelor's or higher degree) and we employed a robust variance estimator to account for the inverse probability weighting.[27] In the analyses of childhood farm exposures, we additionally adjusted for family household income during childhood (poor, low income, middle income, well off).

We conducted two sensitivity analyses. First, we repeated the analyses additionally adjusting for body mass index (BMI) (continuous) and current hormonal contraceptive use (yes, no) at enrollment that may affect measured levels of AMH in adulthood,[28,29] and smoking status at enrollment (never, former, current), history of premenopausal unilateral oophorectomy or partial removal of ovary (yes, no), and history of premenopausal hysterectomy (yes, no), factors well-established to affect ovarian aging.[30–32] We did not adjust for hormone replacement therapy due to concerns that its use may be related to perimenopausal symptoms from ovarian aging in women ages 35–54 years; only 3% of participants reported using hormone replacement therapy. Second, we employed a stricter exposure definition for maternal residence or work on a farm; we considered those who responded "definitely" as exposed and "definitely not" as unexposed.

Since one strategy to re-use data from a case–control study for another outcome is to perform the analyses only among controls, we conducted an exploratory analysis among the nested premenopausal controls (n=866). We were interested in understanding the associations that would have been observed had we not included postmenopausal women in the study sample nor accounted for the matching that was performed in the case–control study.

Results

The distributions of participant characteristics in the weighted sample (n=4,810) and the entire Sister Study cohort meeting the eligibility criteria for the present analyses (n=17,799) were similar (eTable 1).

The median AMH concentration at enrollment in the weighted sample was 0.07 ng/ml (IQR: <LOD-0.59). The median AMH concentration decreased substantially with age (Table 1). Across participant characteristics, we observed median AMH concentration below the LOD among those aged 50–54 years, identifying as Hispanic, current smoking, never consuming alcohol, currently using hormone replacement therapy, with history of premenopausal unilateral oophorectomy or partial ovary removal, with history of premenopausal hysterectomy, with lower childhood family income, and with childhood food insecurity. Participants prenatally exposed to the farm environment less frequently had mothers who smoked while pregnant and tended to have mothers who were older at birth, to have been breastfed, and to have had a lower family income during childhood compared to unexposed participants. Of those prenatally exposed to maternal work or residence on a farm, 71% resided on a farm during childhood. Participants who resided on a farm during childhood more often identified as non-Hispanic black, had completed some college or an Associate's/technical degree, had a lower household income, and had a BMI 25.0–<30.0.

Participants prenatally exposed to maternal work or residence on a farm had lower concentrations of AMH in adulthood compared to unexposed participants (HR 0.66, 95% CI: 0.48–0.90) (Table 2). We did not observe associations between childhood farm residence or pesticide use on crops during childhood farm residence and AMH. Personal handling of crop pesticides and being present in fields during pesticide application during childhood farm residence were only weakly associated with lower AMH concentrations and the HR estimates were accompanied by wide CIs. There was the suggestion of an association between contact with pesticide-treated livestock or buildings during childhood farm residence and lower AMH concentrations in adulthood (HR 0.69, 95% CI: 0.40–1.2).

In our sensitivity analyses in which we (1) additionally adjusted for BMI, current hormonal contraceptive use, smoking status, history of unilateral oophorectomy, and history of hysterectomy, and (2) employed a stricter exposure definition for maternal residence or work on a farm, we observed associations similar to those of the main analyses (eTables 2 and 3).

In our exploratory analyses, premenopausal nested controls (n=866) were more likely to be ages 45–49 years, enrolled earlier into the Sister Study cohort, and non-Hispanic

white compared to the weighted sample (eTable 1). When we repeated the analyses only among the premenopausal nested controls, the estimates of association between prenatal and childhood residence farm exposures and AMH were attenuated compared to estimates from our main analyses using the weighted sample (eTable 4). For example, we observed a HR of 0.79 (95% CI: 0.60–1.0) for the association between prenatal exposure to maternal farm work or residence on a farm and AMH among premenopausal nested controls, compared to HR 0.66, 95% CI: 0.48–0.90 in the weighted sample.

Discussion

We observed that prenatal exposure to the farm environment, and possibly aspects of childhood farm residence, were associated with lower anti-Müllerian hormone concentrations in adulthood. The prenatal and postnatal childhood periods are critical windows of susceptibility for the ovary. During the prenatal period, there is substantial attrition of germ cells during folliculogenesis; some data suggest that as many as ten germ cells are lost for every primordial follicle formed.[33] The pool of primordial follicles substantially declines both before birth and postnatally during childhood due to activation of follicles and atresia.[3,4] Thus, during these periods exposure to ovarian toxicants could contribute to increased follicle attrition, by decreasing the size of the initial prenatal ovarian reserve and/or increasing the rate of atresia.

Our results were inconsistent with another study conducted within the same cohort that focused on menopausal status. That study, conducted among women ages 35–59 years at enrollment, did not observe an association between prenatal exposure to maternal residence or work on a farm and age at natural menopause.[34] The discrepant results may reflect the difference in study outcome. AMH provides a biologic measure of ovarian reserve in premenopausal women, among whom the decline in AMH appears to accelerate after age 40 until approximately age 55, with the greatest decline seen during ages 45–50 years.[35] Thus, it is possible that the use of this ovarian reserve biomarker may be more sensitive than the dichotomous menopausal status for detecting an association. In addition, retrospective recall of age at natural menopause is susceptible to misclassification[36] and cannot be determined in women using hormone replacement therapy or oral contraceptives, or who have undergone hysterectomy.[37]

Our results for prenatal farm exposure and overall childhood farm residence in relation to AMH are similar to our previous findings in a cross-sectional analysis of 1600 premenopausal African-American women ages 23–35 years enrolled in the Study of Environment, Lifestyle & Fibroids (SELF).[6] In SELF, data on maternal farm residence and work were collected using questions worded similarly to those of the Sister Study. After applying the reverse-scale Cox regression analysis to the data in SELF, the estimate of association for maternal residence/work on a farm was HR 0.68 (95% CI: 0.45–1.02, adjusting for age, age squared, and participant education; n=1500 with available data). This estimate is very similar to the estimate in the present study (HR 0.66). However, in SELF, only two questions on childhood farm residence were asked and neither were specific to pesticide exposure: whether the participant lived on a farm or visited a farm for at least one month or longer at any time before age 18 and duration of farm residence. No association

was observed with childhood farm residence (HR 1.14, 95% CI: 0.91–1.43, adjusting for age, age squared, participant education, and childhood household income; n=1600).

One aspect of early-life farm exposure that may affect ovarian reserve is pesticide exposure. Although little is known about the effects of pesticide exposure during childhood on adult ovarian reserve, prenatal exposure to specific pesticides in *in vivo* and *in vitro* laboratory studies have resulted in ovarian germ cell and primordial follicle loss,[13,14] which would decrease the ovarian reserve. An association between prenatal pesticide exposure and lower ovarian reserve has also been observed in human studies. Maternal occupational exposure to pesticides during the first trimester of pregnancy in a prospective study of pregnant greenhouse workers in Denmark was associated with lower AMH concentrations (24% lower, 95% CI: 6%−38%) in daughters ages 6–11 years compared to a reference population of Danish girls.[9] Another Danish pregnancy cohort study, which evaluated maternal concentrations of organochlorine pesticides in serum collected at 30 weeks gestation, reported associations between concentrations of a metabolite of DDT (*p,p'*-DDE) and hexachlorobenzene (HCB) and lower antral follicle counts in a subset of 20 year-old daughters who were non-users of hormonal contraceptives (n=43).[8] In our study we were not able to investigate specific pesticides or aspects of prenatal farm exposure that may affect the ovarian reserve.

In addition, our study was limited by having only a single measurement of serum AMH concentration. Repeated measurements of AMH over time would have allowed us to evaluate early-life farm exposure in relation to the rate of ovarian reserve decline. In addition, had we been able to prospectively follow all Sister Study participants starting in their mid-thirties, we would have been able to more fully capture the premenopausal rate of ovarian reserve decline in women who were postmenopausal at enrollment in the Sister Study cohort.

Our study may have also been limited by relying on participant recall of early-life farm exposure, particularly maternal residence or work on a farm before participants were born. However, 71% of participants reporting maternal residence or work on a farm also resided on a farm during childhood. In addition, the results of a validation study conducted among mothers of 1802 participants suggest the receipt of input from family members when completing the family history questionnaire; there was high sensitivity (83%) and specificity (97%) between the participant and her mother on the reporting of maternal residence or work on a farm (A. D'Aloisio, personal communication, 2 February 2020).

Although our study was limited by the measurement of AMH only among a subset of the Sister Study cohort, a key strength of our study was the ability to leverage available data to allow for inference based on the entire Sister Study cohort ages 35–54 years. Specifically, we included postmenopausal women for whom early-life farm exposure could have contributed to early ovarian aging. We were also able to reweight for the sampling of nested controls who were matched to breast cancer cases and tended be older at baseline and enrolled earlier into the cohort. We thereby reduced selection bias and increased sample size and power to detect associations that would have been missed had we only used data from nested premenopausal controls.

The present analyses were also strengthened by the use of an analytic method that could handle the substantial proportion of observations (41%) with AMH values below the limit of detection.[26] When more than 10% of values are below the detection limit, the use of substitution (using a single value such as LOD/2 or LOD/√2), a common approach to handling undetectable values, can produce marked bias.[38,39] Dichotomizing data based on detection would have decreased power and produced less interpretable parameter estimates.[39] In contrast, the reverse-scale Cox-regression-based approach used in the present analyses has been shown in simulations to maintain coverage for nominal 95% confidence intervals even when greater than 50% of the values are undetectable.[26]

In conclusion, replication of the prenatal farm exposure and lower adult AMH association is consistent with the hypothesis that early-life farm exposures reduce adult ovarian reserve. Further investigation into specific farm exposures, such as pesticides, that may reduce the ovarian reserve is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Distribution of characteristics among participants with and without prenatal farm exposure and childhood farm residence, as well as median AMH concentration for each participant characteristic, Sister Study, 2003–2009.

	Maternal farm residence or work		Childhood farm residence		Enrollment
Characteristic	Yes n=620 [a] (%)[d]	No n=3992 [a] (%)[d]	Yes n=715[b] (%)[d]	No n=4092[b] (%)[d]	AMH (ng/ml) n=4,810[c] Median (IQR)
At enrollment					
Age (years)					
35–39	5	9	5	8	1.97 (0.94–3.21)
40–44	15	17	24	18	0.91 (0.28–1.85)
45–49	35	35	34	35	0.15 (<LOD-0.45)
50–54	46	39	37	39	<LOD (<LOD–<LOD)
Enrollment year					
2003–2006	51	54	47	52	0.08 (<LOD-0.69)
2007–2009	49	46	53	48	0.06 (<LOD-0.46)
Race					
Non-Hispanic white	77	85	69	84	0.07 (<LOD-0.59)
Non-Hispanic black	14	8	22	9	0.14 (<LOD-1.01)
Hispanic	6	5	4	5	<LOD (<LOD-0.38)
Other	3	2	5	2	0.06 (<LOD-0.66)
Missing[e]	0	0.01	0	0	
Education					
≤HS/GED	15	14	12	14	0.03 (<LOD-0.34)
Some college, Associate's, technical degree	39	30	46	29	0.07 (<LOD-0.57)
College graduate or more	46	56	42	57	0.08 (<LOD-0.69)
Missing[e]	0	0.01	0	0	
Total household income (US$)					
<$50,000	19	18	28	19	0.07 (<LOD-0.58)
$50,000–<$100,000	44	40	42	39	0.06 (<LOD-0.52)
≥$100,000	37	42	30	42	0.08 (<LOD-0.57)
Missing[e]	2	2	1	3	
Smoking status					
Never	63	59	69	59	0.10 (<LOD-0.84)
Former	26	32	22	32	0.01 (<LOD-0.29)
Current	11	9	8	9	<LOD (<LOD-0.44)
Missing[e]	0	0.01	0	0	
Alcohol consumption					
Never	6	3	3	3	<LOD (<LOD-0.81)
Former	15	12	11	12	0.01 (<LOD-0.36)
Current	79	86	86	85	0.08 (<LOD-0.66)

Characteristic	Maternal farm residence or work		Childhood farm residence		Enrollment
	Yes n=620 [a] (%)[d]	No n=3992 [a] (%)[d]	Yes n=715[b] (%)[d]	No n=4092[b] (%)[d]	AMH (ng/ml) n=4,810[c] Median (IQR)
Missing[e]	0	0.04	0.04	0.03	
BMI (kg/m^2)					
<25.0	39	44	35	45	0.08 (<LOD-0.79)
≥25.0 – <30.0	34	26	40	26	0.10 (<LOD-0.64)
≥30.0 – <35.0	17	15	15	14	0.01 (<LOD-0.42)
≥35.0	10	14	10	14	0.05 (<LOD-0.27)
Missing[e]	0	0.07	0	0.07	
Parity (number of stillbirths and live births)					
0	20	22	19	21	0.02 (<LOD-0.47)
1	17	15	11	15	0.01 (<LOD-0.50)
2	36	42	46	41	0.09 (<LOD-0.71)
≥3	27	22	25	23	0.07 (<LOD-0.64)
Missing[e]	0	0.11	0	0.11	
Current hormonal contraceptive use					
No	92	90	91	91	0.06 (<LOD-0.58)
Yes	8	10	9	9	0.33 (0.01–1.38)
Missing[e]	2	3	2	3	
Current hormone replacement therapy use					
No	97	96	98	96	0.08 (<LOD-0.63)
Yes	3	4	2	4	<LOD (<LOD–<LOD)
Missing[e]	0.67	0.45	0.08	0.52	
History of premenopausal unilateral oophorectomy or partial ovary removal					
No	93	95	95	95	0.08 (<LOD-0.62)
Yes	7	5	5	5	<LOD (<LOD-0.20)
Missing[e]	0	0.01	0.04	0.01	
History of premenopausal hysterectomy					
No	88	89	90	89	0.08 (<LOD-0.68)
Yes	12	11	10	11	<LOD (<LOD-0.24)
Menopausal					
No	73	80	80	80	0.19 (<LOD-0.92)
Yes	27	20	20	20	All values <LOD
Early-life period					
Maternal smoking					
No	74	62	72	62	0.06 (<LOD-0.48)
Yes	26	38	28	38	0.05 (<LOD-0.43)
Missing[e]	2	3	15	7	

Characteristic	Maternal farm residence or work		Childhood farm residence		Enrollment
	Yes n=620 [a] (%)[d]	No n=3992 [a] (%)[d]	Yes n=715[b] (%)[d]	No n=4092[b] (%)[d]	AMH (ng/ml) n=4,810[c] Median (IQR)
Maternal age (years)					
<20	3	5	4	4	0.10 (<LOD-0.27)
20–34	65	76	72	75	0.06 (<LOD-0.50)
≥35	32	19	24	20	0.06 (<LOD-0.58)
Missing[e]	0.4	1.5	14	5	
Breastfed					
No	46	64	54	62	0.10 (<LOD-0.58)
Yes	54	36	46	38	0.01 (<LOD-0.33)
Missing[e]	7	5	19	8	
Childhood family income					
Poor	14	3	8	4	<LOD (<LOD-0.44)
Low income	41	21	30	21	0.02 (<LOD-0.32)
Middle income	44	66	57	64	0.08 (<LOD-0.70)
Well off	1	10	4	11	0.19 (<LOD-2.43)
Missing[e]	0.64	0.04	0.44	0.03	
Times during childhood when family did not have enough to eat					
No	85	92	89	91	0.08 (<LOD-0.62)
Yes	15	8	11	9	<LOD (<LOD-0.34)
Missing[e]	0	0.02	0.04	0.01	

Abbreviations: AMH, Anti-Müllerian hormone; BMI, body mass index; GED, general equivalency diploma; HS, high school; IQR, interquartile range; LOD, limit of detection.

[a]Unweighted sample size; 11% (weighted) of participants reported that her mother worked or resided on farm while pregnant with participant; 89% (weighted) of participants reported her mother neither worked nor resided on farm while pregnant with participant.

[b]Unweighted sample size; 15% (weighted) and 85% (weighted) of participants reported childhood farm residence and no childhood farm residence, respectively.

[c]Unweighted sample size.

[d]Weighted percent among non-missing.

[e]Weighted percent missing among total.

Table 2.

HRs and 95% CIs for associations between prenatal and childhood farm exposures and AMH concentrations among participants ages 35–54 years (unweighted, n=4,810), Sister Study, 2003–2009.

	n[a]	%[b]	AMH (ng/ml) Median (IQR)	HR (95% CI)[c]
Prenatal farm exposure				
Maternal farm residence or work				
No	3992	89	0.07 (<LOD, 0.54)	1.00 (reference)
Yes	620	11	<LOD (<LOD, 0.32)	0.66 (0.48–0.90)
Missing[d]	198			
Childhood farm exposures				
Farm residence anytime from birth to age 18 for ≥12 months				
No	4092	85	0.07 (<LOD, 0.58)	1.00 (reference)
Yes	715	15	0.08 (<LOD, 0.75)	0.95 (0.74–1.2)
Missing	3			
Pesticide use on crops				
No childhood farm residence	4092	87	0.07 (<LOD, 0.58)	1.00 (reference)
No crops/No crop pesticide use	163	3	0.01 (<LOD, 0.58)	0.96 (0.59–1.5)
Crop pesticide use	445	10	0.12 (<LOD, 1.01)	1.00 (0.76–1.3)
Missing[e]	110			
Personal pesticide handling				
No childhood farm residence	4092	87	0.07 (<LOD, 0.58)	1.00 (reference)
No crops/No crop pesticide use	163	3	0.01 (<LOD, 0.58)	0.96 (0.59–1.5)
Crop pesticide use, no handling	368	8	0.14 (<LOD, 1.01)	1.04 (0.78–1.4)
Crop pesticide use, pesticide handling	72	2	0.12 (<LOD, 0.75)	0.85 (0.48–1.5)
Missing[e]	115			
Present in fields during pesticide application				
No childhood farm residence	4092	87	0.07 (<LOD, 0.58)	1.00 (reference)
No crops/No crop pesticide use	163	3	0.01 (<LOD, 0.58)	0.95 (0.59–1.5)
Crop pesticide use, not present	281	7	0.22 (<LOD, 1.01)	1.05 (0.78–1.4)
Crop pesticide use, present	139	3	0.01 (<LOD, 0.46)	0.88 (0.48–1.6)
Missing[f]	135			
Livestock contact and pesticide use[g]				
No childhood farm residence	4092	86	0.07 (<LOD, 0.59)	1.00 (reference)
No livestock/No livestock contact	248	7	0.25 (<LOD, 1.01)	1.2 (0.88–1.5)
Livestock contact, no pesticide use	244	4	0.02 (<LOD, 0.45)	0.94 (0.60–1.5)
Livestock contact, pesticide use	127	3	0.01 (<LOD, 0.33)	0.69 (0.40–1.2)
Missing[h]	99			

Abbreviations: AMH, Anti-Müllerian hormone; CI, confidence interval; HR, hazard ratio; IQR, interquartile range; LOD, limit of detection.

[a]Unweighted n.

[b] Weighted percent among non-missing.

[c] Adjusted for exact age at blood draw (continuous), age squared (continuous), and education (≤high school/GED, some college/Associate or technical degree, Bachelor's or higher degree). Analyses of childhood farm residence exposures additionally adjusted for family household income during childhood (poor, low income, middle income, well off).

[d] Missing comprises 7% of total weighted sample. Data collected using the self-administered family history questionnaire; data missing for 5% of weighted sample who did not complete the questionnaire (n=90 unweighted).

[e] Missing comprises 2% of the total weighted sample and includes 3 participants (unweighted) reporting "don't know" if farm included crops and 99 participants (unweighted) reporting "don't know" on pesticide use on crops.

[f] Missing comprises 3% of the total weighted sample and includes 3 participants (unweighted) reporting "don't know" if farm included crops and 99 participants (unweighted) reporting "don't know" on pesticide use on crops.

[g] Ever use of pesticides on livestock animals or the buildings where livestock were kept.

[h] Missing comprises 2% of total weighted sample and includes 3 participants reporting "don't know" if farm had livestock and 90 participants reporting "don't know" on pesticide treatment of livestock animals or the buildings where livestock were kept.