HHS Public Access

Author manuscript

Epidemiology. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Epidemiology. 2015 May ; 26(3): 429–435. doi:10.1097/EDE.0000000000000265.

Antimüllerian Hormone and Lifestyle, Reproductive, and Environmental Factors among Women in Rural South Africa

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Abstract

Background—Few data exist regarding antiMüllerian hormone, a marker of ovarian reserve, in relation to environmental factors with potential ovarian toxicity.

Methods—This analysis included 420 women from Limpopo, South Africa studied in 2010–2011. Women were administered comprehensive questionnaires, and plasma concentrations of antiMüllerian hormone and DDT were determined. We used separate multivariable models to examine the associations between natural log-transformed antiMüllerian hormone concentration (ng/ml) and each of the lifestyle, reproductive, and environmental factors of interest, adjusted for age, body mass index, education, and parity.

Results—The median age of women was 24 years (interquartile range [IQR]=22 to 26); the median antiMüllerian hormone concentration was 3.1 ng/ml (IQR=2.0 to 6.0). Women who reported indoor residual spraying in homes with painted walls (indicative of exposure to pyrethroids) had 25% lower (95% confidence interval [CI]=−39% to −8%) antiMüllerian hormone concentrations compared with women who reported no spraying. Little evidence of decreased antiMüllerian hormone concentrations was observed among women with the highest DDT levels.

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Competing Financial Interests: None

Compared with women who used an electric stove, no association was observed among women who cooked indoors over open wood fires. The findings also suggested lower antiMüllerian hormone concentrations among women who drank coffee (−19% [95% CI=−31% to −5%]) or alcohol (−21% [95% CI=−36% to −3%]).

Conclusions—These are among the first data regarding antiMüllerian hormone concentrations relative to pesticides and indoor air pollution. Our results are suggestive of decreased ovarian reserve associated with exposure to pyrethroid pesticides, which is consistent with laboratory animal data.

Anti-Mullerian hormone is a peptide growth factor that was first recognized for its effects on sex differentiation in the male fetus.[1] In adult women, antiMüllerian hormone is produced by granulosa cells and is a marker of ovarian reserve.[2] Animal studies indicate that antiMüllerian hormone inhibits the recruitment of new follicles from the primordial follicle pool and is involved in regulating the number of growing follicles, and selecting follicles for ovulation.[3] Data suggest that women have a fixed ovarian reserve, starting with approximately 1–2 million follicles at birth, after which oocyte numbers decline.[4] By menopause, fewer than 1,000 follicles remain.[5] This decline in oocytes mirrors a decline in antiMüllerian hormone concentration, which peaks sometime during late adolescence or early adulthood, thereafter decreasing until antiMüllerian hormone is undetectable among post-menopausal women.[6, 7]

Anti-Mullerian hormone has been extensively studied among infertile women, and its utility in predicting ovarian response in assisted reproductive technology among this population has been well established.[6, 8, 9] Attention has now focused on studying the distribution and determinants of antiMüllerian hormone concentrations in the general population. Although there have been recent appeals to investigators to consider the assessment of antiMüllerian hormone as a primary outcome measure when examining effects of exposures that may target the ovary,[8] few such investigations exist.

The aim of the present study was to investigate environmental factors affecting antiMüllerian hormone concentrations in reproductive-age women in rural South Africa. Environmental effects on anti-Mullerian hormone are plausible; for example, chemotherapy, radiation, and smoking are known to decrease its concentration.[10–14] We were especially interested in the environmental exposure of cooking over open wood fires, due to shared toxic contaminants of cigarette smoke and combustion by-products of biomass fuel burning, and we hypothesized that women who cooked over open wood fires would have lower anti-Mullerian hormone concentrations. Indoor residual spraying for malaria control (using either dichlorodiphenyltrichloroethane [DDT] or pyrethroids) also occurred in some of the study villages. Given the animal studies showing their adverse effects on the ovary,[15–18] we hypothesized that exposure to these pesticides would be associated with decreased antiMüllerian hormone concentrations. In addition, given the paucity of data on antiMüllerian hormone in women other than those seeking treatment at fertility clinics, we also aimed to describe the associations between demographic, lifestyle, and reproductive factors and antiMüllerian hormone concentrations in this South African sample.

Methods

We used data from the South African Study of Women and Babies, a study designed to examine DDT exposures in relation to reproductive health among women living in eight rural villages in the Vhembe District of the Limpopo Province, South Africa. During 2010–2011, 442 women were enrolled. During the study period, indoor residual spraying for malaria control, using either DDT or pyrethroids, was routinely conducted in half of the villages. Indoor residual spraying typically occurs just prior to and throughout the rainy, summer season. Eligible women were 20–30 years old, were not currently using hormonal contraception or an intrauterine device, had regular menstrual periods (unless currently breastfeeding), had a negative spot pregnancy test, had no previous problems becoming pregnant, and had no medical or other condition that would prevent pregnancy. The present study was approved by institutional review boards of the University of Pretoria, South Africa, and the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health.

Consenting, eligible women were administered a questionnaire regarding demographics, smoking history, alcohol and coffee consumption, reproductive and medical history, self-report of indoor residual spraying, and housing characteristics. Women also completed a short physical exam to obtain anthropometric measurements, including duplicate measures of height and weight at the same visit (which were then averaged), and they provided a blood sample at this time. Among the 442 women initially enrolled, 15 were later found to be ineligible due to age (n=3) or residence outside the study villages (n=12). In addition, a blood sample could not be obtained from one otherwise eligible woman, leaving 426 women eligible for the present analysis.

Both p,p’-DDT and p,p’-dichlorodiphenyldichlorethylene (DDE) concentrations were measured from 2 mL of plasma by the Institute National de Sante Publique du Quebec, in Sainte-Foy, Quebec, Canada using gas chromatography-mass spectrometry. The specific analytic methods, including quality assurance/quality control (QA/QC) are described elsewhere.[19] Briefly, DDT and DDE specimens were analyzed in 14 batches, thirteen of which contained an aliquot from a single QA/QC specimen. The between-batch coefficient of variation for DDT was 5.0% and for DDE was 7.8%. Among the 426 eligible women, DDT values were below the limit of quantification (0.02 μg/L) for four women, and there were assigned a value of one half this value. No values of DDE were below the limit of quantification (0.02 μg/L). Triglycerides (mg/dL) and total cholesterol (mg/dL) were also measured in the plasma samples and were used to estimate total lipids (mg/dL) using the equation: Total Lipids = 1.3×(Triglycerides + Total Cholesterol) + 90 mg/dL.[20]

AntiMüllerian hormone concentrations (ng/ml) were measured in 2012 in plasma (stored in EDTA tubes) using the Gen II enzyme-linked immunosorbent assay (ELISA) from Beckman-Coulter, Inc. (Chaska, MN, USA) at the National Institute of Environmental Health Sciences. Because this assay was designed to measure antiMüllerian hormone in serum samples, we first analyzed paired serum and plasma samples (stored in EDTA tubes) from 11 anonymous subjects from the National Institute of Environmental Health Sciences Clinical Research Unit. This sample served as a validation subset to compare antiMüllerian

hormone concentrations between the two media (serum vs. plasma). The antiMüllerian hormone assay was run in duplicate and averaged for each of these 11 paired samples (for a total of 22 individual measurements) and read within both 30 and 60 minutes. For three of the women, antiMüllerian hormone concentrations were below the detection limit, leaving 8 sets of paired samples (16 individual measurements) for analysis.

For the 426 women in the present study, antiMüllerian hormone was measured in duplicate, and averaged. The within-batch coefficient of variation was 3.8% and the between-batch coefficient of variation was 10.3%. Because antiMüllerian hormone concentrations were skewed with a long tail to the right, we used the natural-log transformed values for all analyses. For one woman, the antiMüllerian hormone concentration was below the limit of detection, leaving 425 women for analysis.

We had data on both chronological age and years since menarche. Given the high correlation (r=0.9) between these variables, we chose to include only chronological age (as a continuous variable) in the analyses. Given the consistently documented decline in antiMüllerian hormone concentrations with increasing age, using linear regression we first examined the age-adjusted associations between antiMüllerian hormone and the following variables: body mass index (BMI; kg/m^2), marital status (not married, married/cohabitating), monthly family income (<1250, 1250–1999, 2000–3000, >3000 Rand), years of education (< grade 11, grade 11, grade 12, > grade 12), parity (nulliparous, 1, >1), total breastfeeding (0, 1–17, 18–27, ≥28 months), current breastfeeding (yes, no), regular coffee consumption (i.e. “at least once per week for six months or longer”; yes, no), ever consumed alcohol (yes, no), passive smoking (i.e. in the past 12 months, has anyone smoked at least one cigarette/day for six months or more near you?; yes/no), cooking fuel (electricity; open wood fire, mostly outside; open wood fire, mostly inside), DDT concentration (μg/L), and DDE concentration (μg/L). In homes with painted walls, indoor residual spraying with pyrethroids is preferred to spraying with DDT, as DDT does not readily absorb into painted surfaces and may leave a residue.[21] In order to capture potential pyrethroid versus DDT exposure we created a variable combining information from women’s self-report of indoor residual spraying and whether the walls of the woman’s home were painted; this variable represents indoor residual spraying status with either pyrethroid or DDT pesticides and has the following categories: none, DDT spraying, and pyrethroid spraying.

Reproductive and demographic variables that were associated with antiMüllerian levels at an alpha level of 0.1 in the age-adjusted models were further examined in multivariable models. Each of the lifestyle and environmental factors was also explored in separate multivariable models. Multivariable models for DDT and DDE also included adjustment for total lipids. Given the potential associations between antiMüllerian hormone concentrations and reproductive history, we also conducted sensitivity analyses excluding women who were currently breastfeeding, and women with recent menarche (<5 years). Lastly, given the potential importance of more recent environmental exposures and because, in this study area, indoor residual spraying begins just prior to and occurs throughout the summer months, we conducted a sensitivity analysis examining adjusted associations between pesticide exposure and antiMüllerian hormone concentrations, by season of the blood draw.

Results

The Pearson correlation coefficient between the eight paired observations of antiMüllerian concentrations in serum and plasma was 0.94, indicating a high correspondence between antiMüllerian hormone concentrations measured in these two media. On average, the antiMüllerian hormone concentrations were 2.1 times higher in plasma than in serum.

The median age of women in this study was 24 years and the median body mass index was 24.7 kg/m^2. The median plasma concentrations of DDT and DDE were 0.89 μg/L and 5.3 μg/L, respectively (Table 1). The median concentration of total lipids was 356.5 mg/dL. Women who reported indoor residual spraying with painted walls (classified as having pyrethroid spraying) had lower median DDT concentrations than women who reported indoor residual spraying with no painted walls (classified as having DDT spraying) (1.4 μg/L vs. 2.2 μg/L, respectively). Overall, the median antiMüllerian hormone concentration in plasma was 3.4 ng/ml (IQR = 2.0 to 6.0). We observed a decline in antiMüllerian hormone across the age range (20–30 years), with an estimated 8% decline (95% confidence interval (CI) = −10% to −5%) per year (Table 1).

Associations between the lifestyle, reproductive, and environmental variables and antiMüllerian hormone concentrations are presented in Table 2; the results in this table are adjusted for age, body mass index, parity, and education. Compared with nulliparous women, those with the highest parity had 20% lower antiMüllerian hormone concentrations (95% CI = −39% to 6%). Women who reported drinking coffee had 19% lower (95% CI = −31% to −5%) antiMüllerian hormone concentrations and women who reported ever drinking alcohol had 21% lower (95% CI = −36% to −3%) antiMüllerian hormone concentrations.

Parity and education were highly correlated (spearman rank correlation coefficient = −0.39, $p<0.0001$). When education was dropped from the model, the association between parity and antiMüllerian hormone concentrations was strengthened; compared with nulliparous women, women with one previous birth had 21% lower antiMüllerian hormone concentrations (95% CI = −36 to −3%), and women with more two or more previous births had 30% lower antiMüllerian hormone concentrations (95% CI = −46% to −10%, data not shown).

Of the environmental factors examined, only indoor residual spraying with pyrethroids was associated with antiMüllerian hormone concentrations (Table 2). Compared with women who reported no indoor residual spraying, women whose homes were likely sprayed with pyrethroids had 25% lower antiMüllerian hormone concentrations (95% CI = −39% to −8%). We found the greatest reduction of antiMüllerian hormone concentrations associated with pyrethroid spraying when antiMüllerian hormone was measured during the rainy summer months when indoor residual spraying occurred (data not shown). However the p-value for the cross-product term between season and indoor residual spraying with pyrethroids was 0.29; the cross-product terms for season and DDT and DDE concentrations were ($p=0.10$ and $p=0.26$, respectively. Excluding women who reported current breastfeeding at the time of the baseline questionnaire, or women with recent menarche, did not result in meaningfully different results (data not shown).

Discussion

Not only is the present study among only a few to have examined antiMüllerian hormone in a population outside the infertility clinic setting, but this particular population is of special interest given their environmental exposures. Ours is the first epidemiologic study to examine pesticide exposures in relation to antiMüllerian hormone concentrations. Pesticide spraying for malaria control occurred in the Vhembe District of the Limpopo Province, South Africa during our study. DDT exposure was a main study interest becase, historically, it has been the primary pesticide used in indoor residual spraying, although the use of pyrethroid pesticides is becoming more common. Within the same village, both pesticides can be used, with pyrethroids most often used in western style homes that have painted surfaces due to the residues left on these surfaces by DDT.[21] We assessed pesticide exposure by combining women's self-report of indoor residual spraying for malaria control with information on whether the home had painted walls. We also measured DDT in participants' plamsa. As expected, median DDT concentrations were lowest in women who lived in villages where no spraying occurred, next highest in women who reported indoor spraying with painted walls (sprayed with pyrethroids), and highest in women who reported indoor residual spraying without painted walls (sprayed with DDT). Given other potential routes of exposure (e.g. diet), it is reasonable to expect that women in villages where DDT was used for indoor residual spraying may have higher concentrations of DDT than women from unsprayed villages, even if the woman's own home was not directly sprayed with DDT. We found no indication of an association between antiMüllerian hormone concentrations and DDT exposure (using either the self-reported exposure variable, or plasma concentrations of DDT and DDE). However, we observed decreased plasma antiMüllerian hormone concentrations among women with probable exposure to pyrethroid pesticides through indoor residual spraying. While exposure to pyrethroids has been previously linked with adverse effects on male reproductive health, the potential hazardous effects on women's reproductive health are less well documented in the epidemiologic literature.[22, 23]

The toxicity of pyrethroids to the ovary depends on the specific pesticide.[16] Deltamethrin and cypermethrin are used for indoor residual spraying in the study area.[24] Data on the ovarian toxicity of deltamethrin are not available, to our knowledge. Cypermethrin, at high doses, reduced ovarian weight in adult rats.[18] Another pyrethroid, bifenthrin, has a number of adverse effects on ovarian cells *in vitro*, including changes in regulation of genes and enzyme activity that control progesterone production and secretion.[17] Fenvalerate, in similar studies, inhibited follicular growth and also decreased production of steroid hormones, including progesterone.[25] Additional experimental studies, with lower doses, of the ovotoxicity of deltamethrin and cypermethrin, with mechanistic data, would inform assessment of the biologic plausibility of the association found in the present study.

Another goal of the present study was to examine ovarian reserve among rural, black South African women in relation to exposure to cooking over open wood fires. Indoor wood-fire smoke is known to increase risk of respiratory problems, but we are unaware of any studies to investigate effects on reproductive outcomes. We observed little evidence of a relationship between antiMüllerian hormone and exposure to wood smoke. Unfortunately,

we did not have personal measurements of exposure to air pollutants or cigarette smoke, and exposure misclassification in each of these measures likely occurred.

In addition to environmental factors, we had the opportunity to explore the relation between antiMüllerian hormone concentrations and demographic, reproductive, and lifestyle factors in this population. The women in the present study were young, with ages ranging from 20 to 30 years. Although previous studies provide convincing evidence of the relation between older age and lower antiMüllerian hormone concentrations, the precise age at which antiMüllerian hormone first begins to decline is unclear. Some studies suggest that antiMüllerian hormone declines after age 20,[26, 27] while others report that the decline does not begin until the mid-twenties.[28, 29] Our results indicate an overall decline in antiMüllerian hormone throughout the 20s among rural, black South African women, a subgroup previously unstudied. Consistent with previous studies,[11, 30] we found no association between body mass index and antiMüllerian hormone concentrations.

Given the positive association between fecundability and gravidity or parity, it might be expected that women with higher parity may also have higher antiMüllerian hormone concentrations. However, epidemiologic studies among the general population have largely failed to support this potential association.[11, 30] We found somewhat lower antiMüllerian hormone among women with higher parity. However, including education in the model may have resulted in overadjustment for parity, given the correlation between the two variables. The association between parity and antiMüllerian hormone concentrations was strengthened after dropping education from the model. Bragg et al.[31] also reported lower antiMüllerian hormone concentrations among women with higher parity when studying young Filipino women (ages 20–22). Because women with polycystic ovary syndrome have high antiMüllerian hormone concentrations and decreased fertility, it is possible that including numerous women with this syndrome in the sample could have resulted in a negative association between antiMüllerian hormone and parity. However, such inclusion was highly unlikely in the present study because women with irregular menstrual cycles or with previous difficulty becoming pregnant were excluded, which would eliminate most women with polycystic ovary syndrome.

We found that women who reported drinking coffee regularly for at least six months, as well as women who report ever consuming alcohol, had lower antiMüllerian hormone concentrations. Although each of these factors was crudely assessed in the present study, our results are consistent with some previous reports of decreased fecundability among women who consume specific types of caffeinated beverages, as well as among women who consume alcohol.[32, 33] The few studies that have examined either of these factors in relation to antiMüllerian hormone concentrations have not supported an association.[11, 34, 35] However, the prior studies were in populations that were very different from the rural South African women we studied. The assay for antiMüllerian that we used was relatively new and has since been shown to give results that reflect the technical details of sample handling and processing.[36, 37] These properties are especially important to note in the clinical setting, where a woman's medical treatment may be influenced by her absolute concentration of antiMüllerian. In the present study, we were focused on relative differences in antiMüllerian concentrations. The precision of the assay, both in our study and in others,[36] has been shown

to be quite acceptable, and thus worked well for our purposes. In our substudy comparing levels of in plasma vs. serum, specimens of each type were handled identically, and gave values that were highly correlated, as has been found by others.[38] However, given the sensitivity of the assay to the details of sample processing, the 2-fold higher values in plasma may not be surprising. Although a single measure of AMH has been considered reliable, recent data indicated moderate variation within-woman in concentration across months.[36] Such variability would decrease the ability to detect long-term associations in studies like ours. Although this study may have been strengthened by the measurement of additional reproductive hormones that serve as markers of ovarian reserve, such as follicle-stimulating hormone, it would have been logistically difficult to time the sample collection to early follicular phase days. Because of the exclusion of infertile women or those with irregular cycles, the variability of measured antiMüllerian hormone concentrations may have been limited. However, the IQR (2.0 ng/ml to 6.0 ng/ml) of antiMüllerian hormone concentrations measured among the subjects in this study does not indicate that this was an issue.

The results from the present analysis provide the first data regarding antiMüllerian hormone concentration, a marker of ovarian reserve, among a group of black, South African women exposed to indoor residual spraying for malaria control and indoor air pollution. AntiMüllerian hormone declined with age even among women in their early 20s in this sample of young women. The suggestive associations we observed for lifestyle and environmental factors, particularly related to pyrethroid exposure, need further investigation, and additional investigation of antiMüllerian hormone as a possible biomarker of exogenous effects on the ovary is warranted.

Acknowledgments

Sources of Funding: This research was supported in part by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Environmental Health Sciences (NIEHS).

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Table 1

Age-adjusted associations between demographic, reproductive, and environmental factors and log(antiMüllerian) concentrations (ng/ml), among 425 women in the South African Study of Women and Babies, 2010–2011

	Descriptive statistics[a]	%Change in antiMüllerian[b]	95% CI
Age (years); median (IQR)	24 (22 to 26)	−8	(−10 to −5)
BMI (kg/m^2); median (IQR)	24.7 (21.5 to 28.3)	−1	(−3 to 0)
DDT (μg/L)[c]; median (IQR)	0.89 (0.27 to 2.7)	−1	(−3 to 1)
DDE (μg/L)[c]; median (IQR)	5.3 (1.5 to 11.0)	0.3	(0 to 1)
Married			
No[d]	267 (63)	0	
Yes/Cohabitating	158 (37)	1	(−17 to 17)
Family Income (Rand)			
<1250	106 (25)	0	
1250–1999	106 (25)	11	(−11 to 39)
2000–3000	109 (26)	8	(−13 to 35)
>3000	104 (24)	4	(−17 to30)
Education			
<Grade 11[d]	100 (24)	0	
Grade 11	121 (28)	22	(−2 to51)
Grade 12	131 (31)	39	(12 to72)
>Grade 12	73 (17)	55	(21 to98)
Parity			
0[d]	87 (21)	0	
1	209 (49)	−22	(−37 to−4)
2+	129 (30)	−32	(−47 to−12)
Total Breastfeeding (months)[e]			
0–17[d]	83 (25)	0	
18–27	143 (42)	−6	(−26 to18)
>28	112 (33)	−4	(−27 to27)
Currently Breastfeeding			
No[d]	369 (87)	0	
Yes	56 (13)	10	(−13 to39)
Coffee Drinker			
No[d]	259 (61)	0	
Yes	166 (39)	−13	(−26 to2)
Passive Smoking			
No[d]	330 (78)	0	
Yes	95 (22)	11	(−8 to35)

	Descriptive statistics[a]	%Change in antiMüllerian[b]	95% CI
Ever Drink Alcohol			
No[d]	352 (83)	0	
Yes	73 (17)	−18	(−33 to1)
Cooking Fuel			
Electricity User[d]	214 (50)	0	
Wood User, Mostly Outside	76 (18)	−3	(−22 to21)
Wood User, Mostly Inside	135 (32)	−9	(−24 to9)
Indoor residual spraying[f]			
None[d]	194 (46)	0	
DDT Spraying	125 (32)	−9	(−25 to9)
Pyrethroid Spraying	88(22)	−25	(−39 to−8)

[a] No. (%), unless otherwise indicated

[b] Calculated using the following formula: [exp(β)−1]*100

[c] Regression model is additionally adjusted for total lipids (mg/dL)

[d] Reference category

[e] Among 338 parous women

[f] 18 women missing; variable based on self-report of spraying, and housing construction

Table 2

Associations between demographic, reproductive, lifestyle, and environmental factors and log (antiMüllerian) concentrations (ng/ml), among 420 women in the South African Study of Women And Babies, 2010–2011, adjusted for age, BMI, education, and parity

	%Change in antiMüllerian[a]	95% CI
Age (years)	−6	(−9 to −3)
BMI (kg/m^2)	−1	(−2 to1)
Education		
<Grade 11[b]	0	
Grade 11	15	(−7 to41)
Grade 12	16	(−7 to44)
>Grade 12	28	(−2 to66)
Parity		
0[b]	0	
1	−15	(−32 to6)
2+	−20	(−39 to6)
Coffee Drinker		
No[b]	0	
Yes	−19	(−31 to−5)
Passive Smoking		
No[b]	0	
Yes	16	(−4 to40)
Ever Drink Alcohol		
No[b]	0	
Yes	−21	(−36 to−3)
Cooking Fuel		
Electricity User[b]	0	
Wood User, Mostly Outside	7	(−14 to33)
Wood User, Mostly Inside	−7	(−22 to11)
Indoor residual spraying[c]		
None[b]	0	
Spraying with DDT	−5	(−22 to13)
Spraying with Pyrethroid	−25	(−39 to−8)
DDT Concentration (μg/L)[d]	−1%	(−3 to1)
DDE Concentration (μg/L)[d]	0.1%	(−0.6 to1)

[a] Calculated using the following formula: [exp(β)−1]*100

[b] Reference category

[c] 18 women missing, based on self-report of spraying, and having painted walls or not

[d] Regression model additionally adjusted for total lipids (mg/dL)