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

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Running Title: AMH concentrations among rural South African Women

Key Words: AMH, environment, fertility, DDT

Acknowledgements: 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).

Competing Financial Interests: None

Abbreviations:

AMH—anti-Mullerian hormone
BMI – body mass index
CI – confidence interval
DDT - dichlorodiphenyltrichloroethane
DDE - dichlorodiphenyldichloroethylene
IQR – interquartile range
LOQ – limit of quantification
NIEHS – National Institute of Environmental Health Sciences
PCOS – Polycystic Ovary Syndrome
TC – total cholesterol
TG – triglycerides
TL – total lipids
QA/QC – quality assurance/quality control

Abstract

Background: Few data exist regarding the distribution and determinants of anti-mullerian hormone (AMH), a marker of ovarian reserve, among populations outside of the fertility clinic setting and few studies have examined AMH concentrations in relation to environmental factors which may have toxic effects on the ovary.

Methods: This analysis included 420 women enrolled in The Study of Women and Babies during 2010-2011, from the Limpopo Province, South Africa. Women were administered questionnaires regarding: demographic, lifestyle, and reproductive factors, indoor residual spraying for malaria control, and housing and cooking characteristics. Concentrations of AMH and DDT were determined from plasma samples. We used separate multivariable models to examine the associations between natural log-transformed AMH concentrations (ng/ml) and each of the lifestyle, reproductive, and environmental factors of interest, adjusted for age, body mass index, and parity.

Results: The median age of women was 24 years (IQR: 22, 26) and the median AMH concentration was 1.6 ng/ml (IQR: 1.0, 2.7). We observed lower AMH concentrations among women with more than two previous births (-29%, 95% CI: -45, -8), and women who drank coffee (-14%, 95% CI: -27, 1%) or alcohol (-18%, 95% CI: -33, 2%). No association with AMH was observed among women who reported cooking over open wood fires. Although little evidence of decreased AMH concentrations were observed among women with the highest DDT or DDE concentrations, women who reported indoor residual spraying in homes with painted walls (likely indicative of exposure to pyrethroid pesticides) had 27% lower (95% CI: -41, -11) AMH concentrations compared with women who reported no indoor residual spraying.

Conclusions: These results provide the first data regarding AMH concentrations in relation to several environmental factors, including pesticides and indoor air pollution. The suggestive associations we observed, particularly related to pyrethroid exposure, need further investigation. Additional study of AMH as a possible biomarker of exogenous effects on the ovary is warranted.

Background:

Anti-Mullerian hormone (AMH) is a peptide growth factor that was first recognized for its effects on sex differentiation *in utero*. In women, AMH is produced by the granulosa cells of small antral and preantral follicles and is a marker of ovarian reserve [1]. Data from animal studies indicate that AMH 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 [2]. Data suggest that women have a fixed ovarian reserve, starting with approximately 1-2 million follicles at birth, after which oocyte numbers decline through follicular atresia and apoptosis, and as a result of ovulation [3]. By menopause, follicle numbers have been exhausted to less than 1,000 [4]. This decline in oocytes mirrors a decline in AMH concentration, which peaks sometime during late adolescence or early adulthood, thereafter decreasing until AMH is undetectable among post-menopausal women [5, 6].

Historically, AMH concentrations have been used to predict ovarian response in assisted reproductive technology [5]. AMH concentrations among infertile women have been examined extensively, and the utility of examining AMH in this population has been well established [7, 8]. Attention has now been focused on studying the distribution and determinants of AMH concentrations in the general population, for which epidemiologic studies have shown an association between low AMH and earlier age at menopause [9-11]. Reports regarding AMH

concentrations and longer times-to-pregnancy (TTP), a traditional measure used in studies of fecundability, have been mixed [12-15], though methods employed vary widely. Despite increasing interest in using AMH to assess reproductive aging in population-based epidemiologic studies, and a push for investigators to consider the assessment of AMH as a primary outcome measure when examining effects of exposures that may target the ovary, epidemiologic research in these areas is scarce [7].

The aim of the present study was to investigate environmental factors affecting AMH concentrations in a sample of reproductive-age women in rural South Africa. Given the number of studies reporting a negative relation between AMH concentrations and cigarette smoking [16-19], 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. Indoor residual spraying for malaria control (using either dichlorodiphenyltrichloroethane (DDT) or pyrethroids) also occurred in some areas of the study. Thus, we had the opportunity to examine pesticide exposures, which are of particular interest based on the mixed reports regarding adverse reproductive effects of DDT and scarce data regarding pyrethroids and women's reproductive health [20, 21]. We collected self-reported data on household spraying as well as housing construction, which likely informs the choice of DDT or pyrethroid in indoor residual spraying [22]. We also measured plasma concentrations of DDT and its primary degradation product and metabolite, dichlorodiphenyldichloroethylene (DDE). Given the paucity of data on AMH in women other than those seeking treatment at fertility clinics, we also describe associations of demographic, lifestyle, and reproductive factors with AMH concentrations.

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 Limpopo Province of 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. 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, and women provided a blood sample. 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.

DDT and DDE concentrations were measured from approximately 2 mL of plasma by the Institute National de Sante Publique du Quebec (INSPQ), in Sainte-Foy, Quebec, Canada using gas chromatography-mass spectrometry. The specific analytic methods are described elsewhere

[23]. Among the 426 eligible women, DDT values were below the limit of quantification (LOQ) (0.02 µg/L) for four women, and were assigned a value of one half the LOQ. No values of DDE were below the LOQ (0.02 µg/L). Triglycerides (TG; mg/dL) and total cholesterol (TC; mg/dL) were also measured in the plasma samples and were used to estimate total lipids (TL; mg/dL) using the equation: TL = 1.3*(TG + TC) + 90 mg/dL [24].

AMH concentrations (ng/ml) were measured in plasma (stored in EDTA tubes) by enzyme-linked immunosorbent assay (ELISA), using the Beckman-Coulter, Inc. (Chaska, MN, USA), AMH ELISA kit. Because this assay was designed to measure AMH in serum samples, we first analyzed paired serum and plasma samples (stored in EDTA tubes) from 11 anonymous subjects from the NIEHS clinical research unit. This sample served as a validation subset to compare AMH concentrations between the two media (serum vs. plasma). The AMH 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 individuals, AMH concentrations were below the detection limit, leaving 8 sets of paired samples (16 individual measurements) for analysis. The Pearson correlation coefficient between the paired sets of observations was 0.9, indicating a high correspondence between AMH concentrations measured in serum and plasma. On average, the AMH plasma concentrations were 2.1 times higher than the serum concentrations.

The AMH concentrations measured among the 426 women in the present study were measured in duplicate, and averaged. We then divided the measurements by 2.1, for comparability to AMH serum concentrations reported in previous studies. The within batch CV was 3.8% and the between batch CV was 10.3%. Because AMH concentrations were skewed with a long tail to the right, we used the natural-log transformed values for all analyses. One

woman had an AMH concentration below the LOD. Additionally, five women reported having ever smoked. Given previous reports of lower AMH among smokers, we chose to exclude these five women from further analysis, leaving 420 women.

We had data on both chronological age as well as years since menarche. Given the high correlation (r=0.9) between these variables, we chose to only include chronological age (as a continuous variable) in the analyses. Given the consistently documented decline in AMH concentrations with increasing age, we first examined, using linear regression, age-adjusted associations between AMH 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), coffee consumption (i.e. 'have you ever drank coffee at least once per week for six months or longer; yes, no), alcohol consumption (i.e. 'have you ever drank 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 user; open wood fire user, mostly outside; open wood fire user, mostly inside), and lipid-adjusted DDT concentration (categorized by quartiles, ng/g), and lipid-adjusted DDE concentration (categorized by quartiles, ng/g). Among women who receive indoor residual spraying, pyrethroids are preferred to DDT in homes with painted walls (DDT may leave a residue because it does not absorb into painted surfaces [22]). In order to capture potential pyrethroid versus DDT exposure we created a variable representing indoor residual spraying status (none, indoor residual spraying in homes without painted walls, and indoor residual spraying in homes with painted walls).

Reproductive and demographic variables which were statistically significant 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 were also explored in multivariable models. Because education was correlated with many of the other variables examined, and may be a proxy for unmeasured factors, adjusting for it may result in over controlling. Therefore, we conducted two sets of multivariable models: one with and one without education. We also conducted sensitivity analyses excluding: (a) women who were currently breastfeeding, and (b) women with recent menarche (<5 years).

Results

The median age of women in this study was 24 years (interquartile range (IQR): 22, 26) and the median BMI was 24.7 kg/m^2 (IQR: 21.5, 28.3) (Table 1). Women tended to be unmarried (63%), with no more than a grade 12 education (83%). Eighty percent were parous, and most parous women had breastfed for 18 months or more. Smoking and alcohol drinking were uncommon. Half of the women in this study reported using electricity to cook foods; of the remaining 210 women who reported cooking with wood, the majority cooked mostly inside the home (n=134, 64%). Among the 211 (52%) women who reported that their home had been sprayed for malaria control, 125 (59%) did not have painted walls and likely received spraying with DDT while 86 (41%) did have painted walls and likely received indoor residual spraying with pyrethroids. The median lipid-adjusted plasma concentrations of DDT and DDE were 238.4 ng/g and 1,534.4 ng/g, respectively. Women who reported indoor residual spraying with painted walls had lower median DDT concentrations than women who reported indoor residual spraying with no painted walls (395.1 ng/g vs. 647.8 ng/g, respectively, data not shown).

Table 1. Age-adjusted associations between demographic, reproductive, and environmental factors and log(AMH) concentrations (ng/ml), among 420 women in the South African Study of Women and Babies, 2010-2011

	Median (IQR)	%Change in AMH[a]	95% CI
Age (years)	24 (22, 26)	-8%	(-10, -5)
BMI (kg/m^2)	24.7 (21.5, 28.3)	-1%	(-3, 0.2)
	n (%)	%Change in AMH	95% CI
Married			
No	264 (63)	reference	
Yes/Cohabitating	156 (37)	-1%	(-16, 17)
Family Income (Rand)			
<1250	105 (25)	reference	
1250-1999	105 (25)	15%	(-8, 44)
2000-3000	109 (26)	10%	(-12, 37)
>3000	101 (24)	4%	(-17, 31)
Education			
<Grade 11	100 (24)	reference	
Grade 11	119 (28)	24%	(-1, 54)
Grade 12	129 (31)	37%	(11, 70)
>Grade 12	72 (17)	55%	(20, 98)
Parity			
0	86 (20)	reference	
1	209 (50)	-21%	(-36, -3)
2+	125 (31)	-30%	(-46, -10)
Total Breastfeeding (months)[b]			
0-17	82 (25)	reference	
18-27	143 (43)	-8%	(-27, 16)
>28	109 (33)	-6%	(-29, 24)
Currently Breastfeeding			
No	364 (87)	reference	
Yes	56 (13)	10%	(-13, 39)
Coffee Drinker			
No	255 (61)	reference	
Yes	165 (39)	-12%	(-25, 4)
Passive Smoking			
No	327 (78)	reference	
Yes	93 (22)	14%	(-6, 38)
Ever Drink Alcohol			
No	351 (84)	reference	
Yes	69 (16)	-18%	(-34, 2)
Cooking Fuel			
Electricity User	210 (50)	reference	
Wood User, Mostly Outside	76 (18)	-3%	(-22, 21)
Wood User, Mostly Inside	134 (32)	-8%	(-24, 10)
Self Reported indoor residual spraying[c]			
None	191 (48)	reference	
Yes, no painted walls	125 (31)	-10%	(-26, 8)
Yes, with painted walls	86(21)	-28%	(-41, -11)
Lipid Adjusted DDT Concentration (ng/g)			
<78.4	105 (25)	reference	
78.4-238.4	105 (25)	-1%	(-21, 23)
238.5-777.8	106 (25)	-15%	(-32, 6)
>777.8	104 (25)	-15%	(-32, 6)
Lipid Adjusted DDE Concentration (ng/g)			
<441.8	103 (25)	reference	
441.8-1534.4	107 (25)	-14%	(-31, 8)
1534.5-3166.0	106 (25)	-20%	(-36, -0.5)
>3166.1	104 (25)	-2%	(-22, 23)

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

[b]among 334 parous women

[c]18 women missing

Overall, the median AMH concentration was 1.6 ng/ml (interquartile range (IQR): 1.0, 2.7) (data not shown). The median concentrations of AMH by age are shown in Figure 1. There was a statistically significant decline in AMH across the age range (20-30), with an estimated -8% decline (95% Confidence Interval (CI): -10, -5) per year (Table 1). The age-adjusted association between the lifestyle, reproductive, and environmental variables revealed lower AMH concentrations associated with higher BMI, higher parity, positive report of indoor residual spraying in homes with painted walls (likely reflecting pyrethroid rather than DDT spraying), and higher AMH concentrations associated with greater education (Table 1).

We examined these associations with further adjustment for BMI, parity, and education (Table 2, Model 1). Because we were especially interested in the environmental factors, and the exposed women tended to have less education, we dropped control for education in the second set of analyses (Table 2, Model 2). As noted above, this was done to avoid potential over-control and the possible attenuation of associations that could result from it. Education remained positively associated with AMH, even when adjusted for parity. Women with higher parity had lower AMH concentrations, although the strength of the association was attenuated and the association was no longer statistically significant when education was included in the model (Table 2).

Both coffee consumption and alcohol consumption were associated with lower AMH concentrations (Table 2). In the education-adjusted model, women who reported drinking coffee had -18% (95% CI: -31, -4%) lower AMH concentrations and women who reported ever drinking alcohol had -21% (95% CI: -36, -2%) lower AMH concentrations. The estimates were slightly attenuated without control for education (Model 2). Passive smokers tended to have higher AMH concentrations, though not statistically significantly. The analysis for cooking with

wood-fuel inside the home showed an estimated -7% (95% CI: -22, 11%; Model 1) or -9% (95% CI: -24, 8%; Model 2) lower AMH concentrations in the exposed, but the associations were not statistically significant.

We examined potential pesticide exposures using three metrics: indoor residual spraying in the home for malaria control (based on self-report), lipid-adjusted plasma DDT concentrations, and lipid-adjusted plasma DDE concentrations. AMH tended to be lower among exposed women for each of these metrics, especially for indoor residual spraying in homes with painted walls (likely reflecting exposure to pyrethroids). As expected, estimates were stronger when no adjustment for education was included (Table 2, Model 2). AMH concentrations were an estimated 27 - 28% lower among women who reported indoor residual spraying in homes with painted walls compared to women in non-sprayed homes (95% confidence interval -41, -11% for Model 2 and -41, -10% for Model 1). Women with higher lipid-adjusted plasma DDT concentrations had lower AMH concentrations, but the association was not statistically significant even when education was dropped from the model (estimated percent change for the highest quartile compared to lowest was -13%, 95% CI -30, 9%) (Table 2, Model 2). The relation between AMH and DDE concentrations was non-linear across the quartiles and not statistically significant for any of the categories. Nor were associations statistically significant when DDT and DDE were analyzed as linear variables (data not shown). 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).

Table 2. Fully-adjusted associations between demographic, reproductive, lifestyle, and environmental factors and log(AMH) concentrations (ng/ml), among 420 women in the South African Study of Women And Babies, 2010-2011

	Model 1[a]		Model 2[b]	
	%Change in AMH[c]	95% CI	%Change in AMH[c]	95% CI
Age (years)	-6%	(-9, -3)	-6%	(-9, -3)
BMI (kg/m^2)	-1%	(-2, 1)	-1%	(-2, 1)
Education				
<Grade 11	reference		--	--
Grade 11	15%	(-7, 41)	--	--
Grade 12	16%	(-7, 44)	--	--
>Grade 12	28%	(-2, 66)	--	--
Parity				
0	reference		reference	
1	-15%	(-31, 6)	-20%	(-36, -2)
2+	-18%	(-38, 9)	-29%	(-45, -8)
Coffee Drinker				
No	reference		reference	
Yes	-18%	(-31, -4)	-14%	(-27, 1)
Passive Smoking				
No	reference		reference	
Yes	18%	(-2, 43)	14%	(-5, 38)
Ever Drink Alcohol				
No	reference		reference	
Yes	-21%	(-36, -2)	-18%	(-33, 2)
Cooking Fuel				
Electricity User	reference		reference	
Wood User, Mostly Outside	6%	(-14, 32)	1%	(-19, 25)
Wood User, Mostly Inside	-7%	(-22, 11)	-9%	(-24, 8)
Self Reported indoor residual spraying[d]				
None	reference		reference	
Yes, no painted walls	-6%	(-22, 13)	-8%	(-23, 10)
Yes, with painted walls	-27%	(-41, -10)	-28%	(-41, -11)
Lipid Adjusted DDT Concentration, ng/g				
<78.4	reference		reference	
78.4-238.4	2%	(-18, 28)	2%	(-19, 27)
238.5-777.8	-7%	(-26, 16)	-11%	(-29, 11)
>777.8	-9%	(-28, 14)	-13%	(-30, 9)
Lipid Adjusted DDE Concentration, ng/g				
<441.8	reference		reference	
441.8-1534.4	-11%	(-28, 12)	-12%	(-30, 9)
1534.5-3166.0	-15%	(-32, 7)	-18%	(-34, 3)
>3166.1	-2%	(-22, 22)	-4%	(-23, 20)

[a]adjusted for age, BMI, parity, and education

[b]adjusted for age, BMI, and parity

[c]Calculated using the following formula: [exp(β)-1]*100

[d]18 women missing

Discussion

Not only is the present study among only a few to have examined AMH in a population outside the infertility clinic setting, but this particular population is of special interest given their

environmental exposures. Compared to white women in the United States, African American women may experience earlier age at menopause as well as lower age-specific AMH concentrations [25, 26]. Our South African women had even lower AMH concentrations than those reported for African American women (median AMH 1.6 ng/ml vs. 2.2 ng/ml) [24] despite the younger age of our sample (median age of 24 vs. 36). This suggests that black South African women may also experience early menopause, though comparisons of AMH between populations are difficult without assurance of the same sample-collection protocol and laboratory conducting the assay. Little research exists regarding racial disparities in reproductive factors among black South African women, although earlier ages at menopause for blacks compared with white South Africans has been reported [27].

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 AMH concentrations, the precise age at which AMH first begins to decline is unclear. Some studies suggest that AMH declines after age 20 [28, 29], while others report that the decline does not begin until the mid-twenties [15, 30]. Kelsey et al. [30] developed and validated a model of AMH concentrations over a woman's lifespan, using over 3,000 data points from published studies, and the results suggest that AMH peaks at 24.5 years, declining thereafter. Our results indicate an overall decline in AMH throughout the 20s among rural black, South African women, a subgroup previously unstudied. Though our results indicate a small decline in AMH concentration per unit increase in BMI, previous studies among women from the general population have reported no association between AMH and BMI [16, 31].

Given the positive association between fecundability and gravidity or parity, it might be expected that women with higher parity may also have higher AMH concentrations. However,

epidemiologic studies among the general population have largely failed to support this potential association [16, 31]. We found the opposite association to that expected (lower AMH among women with higher parity). Bragg et al. [32] also reported lower AMH concentrations among with higher parity when studying young Filipino women (ages 20-22). Because women with polycystic ovary syndrome (PCOS) have high AMH concentrations and decreased fertility, it is possible that including numerous women with PCOS in the sample could result in a negative association between AMH and parity. However, this is unlikely to be an issue in the present study. Women with irregular menstrual cycles or with previous issues becoming pregnant were excluded, which would also eliminate most women with PCOS.

Few studies have examined the impact of lifestyle factors on AMH in the general population, with the exception of smoking. The epidemiologic literature clearly points to a relation between adverse reproductive outcomes, including fertility and earlier menopause, among active smokers [33]. Age-specific AMH concentrations among smokers are also lower than for non-smokers [16-19]. The reproductive impact of passive smoke exposure is less clear. Although only five women in the present study reported active smoking, and were excluded from the analysis, 22% (n=93) of the women reported exposure to passive smoke. Contrary to expectation, we found that, on average, these women had higher AMH concentrations, though the confidence interval was broad.

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 AMH 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 caffeine as well as among women who consume alcohol [34, 35]. In contrast, the few studies

that have examined either of these factors in relation to AMH concentrations have not supported an association [16, 36, 37]. However, the prior studies were in very different populations from the rural South African women we studied.

One of the main goals of the present study was the examine ovarian reserve among black, South African women in relation to several little-studied environmental exposures, including 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 only a small, nonsignificant negative association between AMH and this exposure. Unfortunately, we did not have personal measurements of exposure to air pollutants, and exposure misclassification likely occurred.

Ours is the first epidemiologic study to examine pesticide exposures in relation to AMH concentrations. Pesticide spraying for malaria control occurred in the Limpopo District of South Africa during our study. Historically, DDT has been the primary pesticide used in indoor residual spraying, although pyrethroids may be used in western style homes that have painted surfaces due to the residues left on these surfaces by DDT [22]. 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. Women who reported indoor residual spraying with painted walls had lower median concentrations of DDT. In regression analyses, lttle indication of lower AMH concentrations were observed among women who reported indoor residual spraying with no painted walls (likely indicative of DDT exposure). However, we did observe a large, statistically significant negative association between AMH concentrations and indoor residual spraying in homes with painted walls, which is suggestive of an adverse effect of pyrethroid exposure on women's reproductive health. 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 [20, 21]. Laboratory animal data suggest ovarian toxicity of pyrethroid pesticides, including adverse effects on follicular development, indicating that our findings may be biologically plausible [38-40].

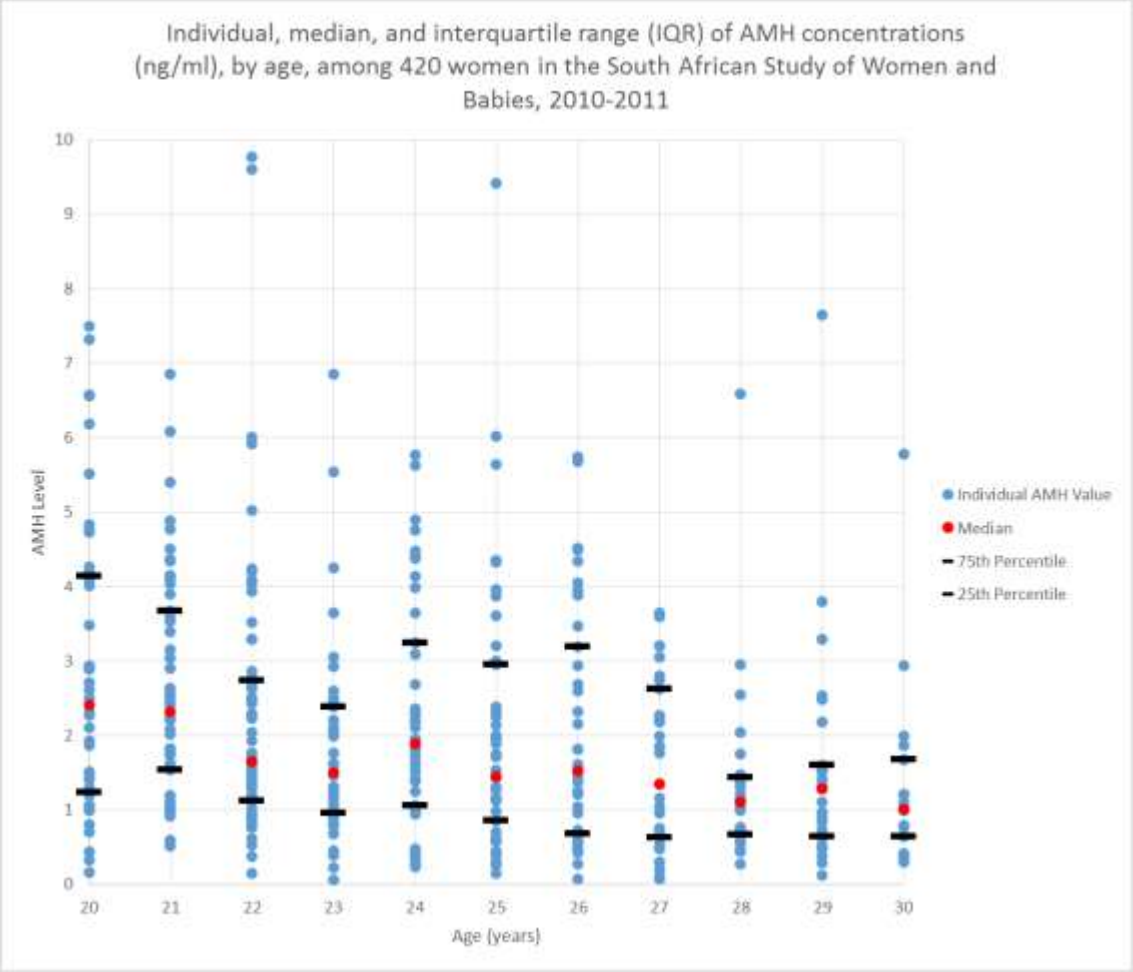
We also assessed DDT exposure by directly measuring plasma concentrations of DDT and its metabolite, DDE. DDT, an organochlorine pesticide classified as an endocrine disruptor, has been detected in follicular fluid [41]. Although a review of toxicologic data indicates the potential for organochlorine pesticides, including DDT, to adversely affect female reproduction [42], epidemiologic studies of these pesticide's effects on women's reproductive health, including indicators of ovarian reserve, have been mixed [43-47]. The associations for plasma DDT and DDE with AMH were not clear. Confidence intervals were broad for both DDT and DDE categories, and there was not a monotonic dose-response pattern for DDE.

In summary, the results from the present analysis provide the first data regarding AMH 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. AMH 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 more study of AMH as a possible biomarker of exogenous effects on the ovary is warranted.

1. Hansen, K.R., et al., *Correlation of ovarian reserve tests with histologically determined primordial follicle number.* Fertility and Sterility, 2011. **95**(1): p. 170-175.
2. Visser, J.A. and A.P. Themmen, *Anti-Mullerian hormone and folliculogenesis.* Mol Cell Endocrinol, 2005. **234**(1-2): p. 81-6.
3. Vaskivuo, T.E., et al., *Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4.* J Clin Endocrinol Metab, 2001. **86**(7): p. 3421-9.
4. Faddy, M.J., et al., *Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause.* Hum Reprod, 1992. **7**(10): p. 1342-6.
5. La Marca, A., et al., *Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART).* Hum Reprod Update, 2010. **16**(2): p. 113-30.
6. Lee, M.M., et al., *Mullerian inhibiting substance in humans: normal levels from infancy to adulthood.* J Clin Endocrinol Metab, 1996. **81**(2): p. 571-6.
7. Baird, D.D. and A.Z. Steiner, *Anti-Mullerian hormone: a potential new tool in epidemiologic studies of female fecundability.* Am J Epidemiol, 2012. **175**(4): p. 245-9.
8. Seifer, D.B., V.L. Baker, and B. Leader, *Age-specific serum anti-Mullerian hormone values for 17,120 women presenting to fertility centers within the United States.* Fertility and Sterility, 2011. **95**(2): p. 747-750.
9. Broer, S.L., et al., *Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women.* J Clin Endocrinol Metab, 2011. **96**(8): p. 2532-9.
10. Freeman, E.W., et al., *Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women.* J Clin Endocrinol Metab, 2012. **97**(5): p. 1673-80.
11. Dolleman, M., et al., *Anti-Mullerian hormone is a more accurate predictor of individual time to menopause than mother's age at menopause.* Hum Reprod, 2014. **29**(3): p. 584-91.
12. Steiner, A.Z., et al., *Antimüllerian hormone as a predictor of natural fecundability in women aged 30-42 years.* Obstetrics and Gynecology, 2011. **117**(4): p. 798-804.
13. Hendriks, A.E.J., et al., *Fertility and ovarian function in high-dose estrogen-treated tall women.* Journal of Clinical Endocrinology and Metabolism, 2011. **96**(4): p. 1098-1105.
14. Streuli, I., et al., *AMH concentration is not related to effective time to pregnancy in women who conceive naturally.* Reprod Biomed Online, 2014. **28**(2): p. 216-24.
15. Hagen, C.P., et al., *Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 turner syndrome patients.* Journal of Clinical Endocrinology and Metabolism, 2010. **95**(11): p. 5003-5010.
16. Dolleman, M., et al., *Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study.* J Clin Endocrinol Metab, 2013. **98**(5): p. 2106-15.
17. Plante, B.J., et al., *The impact of smoking on antimullerian hormone levels in women aged 38 to 50 years.* Menopause, 2010. **17**(3): p. 571-6.
18. Sowers, M.R., et al., *Relating smoking, obesity, insulin resistance, and ovarian biomarker changes to the final menstrual period*, in *Annals of the New York Academy of Sciences*. 2010. p. 95-103.
19. Waylen, A.L., G.L. Jones, and W.L. Ledger, *Effect of cigarette smoking upon reproductive hormones in women of reproductive age: A retrospective analysis.* Reproductive BioMedicine Online, 2010. **20**(6): p. 861-865.
20. Koureas, M., et al., *Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes.* Toxicol Lett, 2012. **210**(2): p. 155-68.
21. Caserta, D., et al., *Environment and women's reproductive health.* Hum Reprod Update, 2011. **17**(3): p. 418-33.

22. Biscoe, M.L., C.M. Mutero, and R.A. Kramer, *Current Policy and Status of DDT use for Malaria Control in Ethiopia, Uganda, Kenya and South Africa*. 2005.
23. Whitworth, K.W., et al., *Predictors of Plasma DDT and DDE Concentrations among Women Exposed to Indoor Residual Spraying for Malaria Control in the South African Study of Women and Babies (SOWB).* Environ Health Perspect, 2014.
24. Rylander, L., P. Nilsson-Ehle, and L. Hagmar, *A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids.* Chemosphere, 2006. **62**(3): p. 333-6.
25. Schuh-Huerta, S.M., et al., *Genetic variants and environmental factors associated with hormonal markers of ovarian reserve in Caucasian and African American women.* Hum Reprod, 2012. **27**(2): p. 594-608.
26. Seifer, D.B., et al., *Variations in serum mullerian inhibiting substance between white, black, and Hispanic women.* Fertil Steril, 2009. **92**(5): p. 1674-8.
27. Frere, G., *Mean age at menopause and menarche in South Africa.* S Afr J Med Sci, 1971. **36**(1): p. 21-4.
28. de Vet, A., et al., *Antimullerian hormone serum levels: a putative marker for ovarian aging.* Fertil Steril, 2002. **77**(2): p. 357-62.
29. Nelson, S.M., et al., *Nomogram for the decline in serum antimÃ¼llerian hormone: A population study of 9,601 infertility patients.* Fertility and Sterility, 2011. **95**(2): p. 736-741.e3.
30. Kelsey, T.W., et al., *A validated model of serum Anti-Mullerian hormone from conception to menopause.* PLoS ONE, 2011. **6**(7).
31. La Marca, A., et al., *Normal serum anti-Mullerian hormone levels in the general female population and the relationship with reproductive history.* Eur J Obstet Gynecol Reprod Biol, 2012. **163**(2): p. 180-4.
32. Bragg, J.M., et al., *Age at menarche and parity are independently associated with Anti-Mullerian hormone, a marker of ovarian reserve, in Filipino young adult women.* Am J Hum Biol, 2012. **24**(6): p. 739-45.
33. Rogers, J.M., *Tobacco and pregnancy.* Reprod Toxicol, 2009. **28**(2): p. 152-60.
34. Sharma, R., et al., *Lifestyle factors and reproductive health: taking control of your fertility.* Reprod Biol Endocrinol, 2013. **11**: p. 66.
35. Hatch, E.E., et al., *Caffeinated beverage and soda consumption and time to pregnancy.* Epidemiology, 2012. **23**(3): p. 393-401.
36. Dalleac, A., et al., *0-084 Caffeine intake does not affect femaile ovarian reserve. Analysis of a cohort of 1963 consecutive blood samples evaluating hormonal ovarian status.* Human Reproduction, 2013. **28 (suppl 1)**: p. i35-i37.
37. Nardo, L.G., et al., *Anti-Mullerian hormone levels and antral follicle count in women enrolled in in vitro fertilization cycles: relationship to lifestyle factors, chronological age and reproductive history.* Gynecol Endocrinol, 2007. **23**(8): p. 486-93.
38. Guerra, M.T., F.C. de Toledo, and G. Kempinas Wde, *In utero and lactational exposure to fenvalerate disrupts reproductive function in female rats.* Reprod Toxicol, 2011. **32**(3): p. 298-303.
39. Liu, J., et al., *Enantioselective endocrine-disrupting effects of bifenthrin on hormone synthesis in rat ovarian cells.* Toxicology, 2011. **290**(1): p. 42-9.
40. Fei, J., et al., *Fenvalerate inhibits the growth of primary cultured rat preantral ovarian follicles.* Toxicology, 2010. **267**(1-3): p. 1-6.
41. Jirsova, S., et al., *Effect of polychlorinated biphenyls (PCBs) and 1,1,1-trichloro-2,2,-bis (4-chlorophenyl)-ethane (DDT) in follicular fluid on the results of in vitro fertilization-embryo transfer (IVF-ET) programs.* Fertil Steril, 2010. **93**(6): p. 1831-6.

42. Tiemann, U., *In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review.* Reprod Toxicol, 2008. **25**(3): p. 316-26.
43. Ouyang, F., et al., *Serum DDT, age at menarche, and abnormal menstrual cycle length.* Occup Environ Med, 2005. **62**(12): p. 878-84.
44. Harley, K.G., et al., *DDT exposure, work in agriculture, and time to pregnancy among farmworkers in California.* J Occup Environ Med, 2008. **50**(12): p. 1335-42.
45. Cohn, B.A., et al., *DDT and DDE exposure in mothers and time to pregnancy in daughters.* Lancet, 2003. **361**(9376): p. 2205-6.
46. Law, D.C., et al., *Maternal serum levels of polychlorinated biphenyls and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and time to pregnancy.* Am J Epidemiol, 2005. **162**(6): p. 523-32.
47. Richardson, M.C., et al., *Environmental and developmental origins of ovarian reserve.* Hum Reprod Update, 2013.


Individual, median, and interquartile range (IQR) of AMH concentrations (ng/ml), by age, among 420 women in the South African Study of Women and Babies, 2010-2011
AMH Level
0
1
2
3
4
5
6
7
8
9
10
20
21
22
23
24
25
26
27
28
29
30
Age (years)
Individual AMH Value
Median
75th Percentile
25th Percentile