Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Grayson Donley Date

Association between *in utero* perfluoroalkyl substance exposure and anti-Müllerian hormone levels in adolescent females

By

Grayson Donley
MSPH

Epidemiology

_____

Terryl J. Hartman, PhD, MPH, RD
Committee Chair

Association between *in utero* perfluoroalkyl substance exposure and anti-Müllerian hormone levels in adolescent females

By

Grayson Donley

Bachelor of Arts, Biology
Kenyon College
2014

Thesis Committee Chair: Terryl J. Hartman PhD, MPH, RD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Epidemiology
2017

Abstract

Association between *in utero* perfluoroalkyl substance exposure and anti-Müllerian hormone levels in adolescent females

By Grayson Donley

Perfluoroalkyl substances (PFAS) are synthetic, ubiquitous chemicals that can cross the placental barrier and impact reproductive health. Lower levels of anti-Müllerian hormone (AMH), a serum marker of ovarian reserve, are associated with reduced fertility. Some evidence indicates that *in utero* environmental exposures could influence reproduction in female offspring through changes in ovarian development and function. We investigated the association between *in utero* PFAS exposure and AMH levels in female adolescents using data from the Avon Longitudinal Study of Parents and Children, a British pregnancy cohort. Maternal serum samples were collected during pregnancy and analyzed for concentrations of four commonly found PFAS, perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA). AMH levels were measured in serum of female offspring (mean age, 15.4 years). We used a sample of 446 mother-daughter dyads for multivariable linear regression analyses, controlling for maternal age at delivery, pre-pregnancy body-mass index, and maternal education. Multiple imputation was utilized to impute missing values of AMH (61.2%) and covariates. Mean PFAS concentrations (ng/mL (SD)) were: PFOS 21.32 (10.13), PFOA 4.02 (1.86), PFHxS 2.43 (4.93), PFNA 0.56 (0.28). AMH levels were log-transformed for analyses. The geometric mean AMH concentration was 3.88 ng/mL (IQR: 2.67, 6.37). After controlling for confounders, mean differences in AMH per one ng/mL increases in PFOA, PFOS, PFHxS, and PFNA were 2.9% (95% CI: -2.3%, 8.2%, p=0.25), 0.5% (95%CI: -0.4%, 1.5%, p=0.24), 0.9% (95% CI: -0.5%, 2.3%, p=0.21), and 8.0% (-76.9%, 92.9%, p=0.82) respectively. These findings suggest that there is no association between *in utero* PFAS exposure and AMH levels in female adolescents.

Association between *in utero* perfluoroalkyl substance exposure and anti-Müllerian hormone levels in adolescent females

By

Grayson Donley

Bachelor of Arts, Biology
Kenyon College
2014

Thesis Committee Chair: Terryl J. Hartman PhD, MPH, RD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment for the requirements for the degree of
Master of Science in Public Health
in Epidemiology
2017

Acknowledgements

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. The UK Medical Research Council and the Wellcome Trust (Grant ref: 092731) and the University of Bristol provide core support for ALSPAC. Further, I would like to thank my professors at Emory University as well as everyone in the Health Studies Branch for teaching me and providing me with invaluable resources to help me complete my analyses. I would also like to express my deepest gratitude to my family who supported me every step of the way. Lastly, I would like to thank my advisors Dr. Terry Hartman and Dr. Ethel Taylor for their continued support and guidance throughout this project.

Table of Contents

Introduction

The Endocrine Society defines an endocrine disrupting chemical (EDC) as "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" (Zoeller et al., 2012). EDCs may alter an individual's physiology at any time point, from fetal development into adulthood (Diamanti-Kandarakis et al., 2015). Researchers have conducted numerous studies on the impact of EDC exposure on human health outcomes including cardiovascular disease, obesity, female and male reproductive health, thyroid disruption, and neurodevelopmental and neuroendocrine effects (Zoeller et al., 2012).

A subset of EDCs known as perfluoroalkyl substances (PFAS) are synthetic ubiquitous chemicals that were widely used in industrial and consumer products, as their high stabilities and low surface tensions make them ideal surfactants (Kissa, 2001; Lau et al., 2007). Common consumer use of PFAS as surfactants include food packaging, non-stick coatings and textile coatings (Lau et al., 2007). Although the main manufacturer phased out production of perfluorooctanoic acid (PFOA) in 2002, PFAS can have a half-life up to 8.5 years in humans (Lau et al., 2007). Further, PFAS accumulate in both the food chain and environment, which leads to potential human exposure through ingestion of contaminated food or drinking water (Fromme et al., 2009). The four most commonly detected PFAS are perfluoroctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), PFOA and perfluorononanoic acid (PFNA; Kato et al., 2011). The United States National Health and Nutrition Examination Survey (NHANES) detected PFOS, PFOA, PFHxS, and PFNA in more than 95% of participants between 1999 and 2008 (Kato et al., 2011).

PFAS can cross the placental barrier into the fetal environment, which raises questions about the effect of prenatal PFAS exposure on the health of offspring (Needham et al., 2011). Exposure to environmental toxins such as PFAS during fetal development can have long-term effects due to the cell proliferation and differentiation that occurs during this time frame (DeWitt, 2015). Previous work by Kristensen et al. suggests that *in utero* PFAS exposure could influence reproduction in female offspring through changes in ovarian development and function (2013).

Anti-Müllerian hormone (AMH), previously referred to as Müllerian Inhibiting Substance, is a member of the transforming growth factor-beta family, which helps regulate the process of ovarian follicle maturation (Weenen et al., 2004). AMH levels are strongly correlated with the number of primordial, or resting, ovarian follicles which form during fetal development and deplete over time (Weenen et al., 2004). Women who experience a faster rate of depletion of the follicle pool are more likely to have a younger age at menopause (te Velde and Pearson, 2002). Previous work found a strong correlation between serum levels of AMH and the number of ovarian primordial follicles, contributing to a body of research that suggests AMH is a biomarker of ovarian reserve in adult and adolescent females (Hansen et al., 2011).

There is some limited evidence based on studies assessing the relationship between PFAS exposure and reproductive outcomes that suggests prenatal exposures may influence daughters' AMH levels later in life. The findings regarding the relationship between prenatal PFAS exposure and age at menarche are inconsistent. In the Avon Longitudinal Study of Parents and Children (ALSPAC), an analysis of 218 female adolescents with early menarche and 230 without early menarche found no association

between prenatal PFAS exposure and early onset age at menarche among offspring (Christensen et al., 2011). Other researchers found no association between prenatal PFOS exposure and age of menarche, but their findings did suggest increased *in utero* PFOA exposure was significantly associated with a 5.3 months later age of menarche in a sample of 377 women in a Danish national birth cohort (Kristensen et al., 2013). In an analysis of 1,743 participants from the Maternal-Infant Research on Environmental Chemicals Study in Canada, researchers concluded exposure to PFOA and PFHxS were associated with a longer time to pregnancy (Vélez et al., 2015).

Previous ALSPAC research with 1,399 daughters ages 14 to 16 years found an inverse association between maternal gestational weight gain and daughters' AMH level. Daughters of women in the top quintile of maternal gestational weight gain had AMH levels 0.83 times the levels in daughters of women in the four lower quintiles of the distribution (Fraser et. al, 2013). In a study of 540 young adults aged 12 to 30 years in Taiwan, Tsai et al. explored the association between serum PFAS concentrations and levels of reproductive hormones, but did not assess AMH (2015). Among female participants ages 12 to 17, researchers concluded that PFOA was associated with decreased sex hormone-binding globulin and perfluorodecanoic acid (PFUA) was associated with decreased follicle-stimulating hormone (FSH) in females (Tsai et al., 2015). The results of this study provide evidence to suggest prenatal PFAS exposure can be associated with reproductive hormone levels in female adolescents.

A Danish national birth cohort found no associations between prenatal PFOS or PFOA exposure and concentrations of reproductive hormones, including AMH, in female offspring approximately 20 years old (Kristensen et al., 2013). To our knowledge, no

study has evaluated the association between prenatal PFAS exposure and AMH levels in adolescent females to date. The aim of this study was to examine the association of prenatal PFAS exposure to PFOA, PFOS, PFxHS, and PFNA with AMH levels in female adolescents aged 14-16 years.

Methods

Population

ALSPAC is an ongoing longitudinal birth cohort, initially established to determine how genetic and environmental characteristics impact health and development in both parents and children (Fraser et al., 2013). Pregnant women in three health districts of the county of Avon, Great Britain were recruited for the study between 1990 and 1992, with additional recruitment phases at the 7 and 8-year follow-up to identify eligible children. A total of 15,247 eligible pregnancies were enrolled in ALSPAC (Fraser et al., 2013). A series of questionnaires and clinical assessments were administered to mothers during pregnancy and continue to be administered to children enrolled in ALSPAC to collect health and demographic information. Detailed ALSPAC study design and recruitment methods have been previously outlined elsewhere (Fraser et al., 2013). Mother-daughter dyads identified for a nested case-control study exploring the associations between endocrine disrupting chemicals and adolescent development were included in the present study. In the ancillary study, case-control status was determined by age at menarche, where early menarche was defined as menarche before 11.5 years and controls were girls who attained menarche at or after 11.5 years of age. We constructed stratum-weighted linear regression models to account for the sampling

selection probabilities where the weight for cases was 1 and the weight for controls was 15.1.

Ethical approval for this study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee. All participants in the study provided written informed consent and parents provided written informed consent for their child.

Anti-Müllerian Hormone Assessment

AMH levels were measured from blood samples taken during a clinical assessment. Participants attending the morning 14-16 year clinic were asked to fast overnight and participants attending the clinic after lunch were asked to fast for a minimum of 6 hours. Blood samples were immediately spun and serum frozen at -80°C. As previously described, AMH was assayed on serum using the commercial AMH Generation II ELISA kit (Beckman Coulter UK Ltd, High Wycombe, United Kingdom) [Wallace et al., 2011). Both the inter- and intra-assay coefficients of variation were less than 5%. AMH values in this study are reported in ng/mL.

Perflouroalkyl Substances

PFOS, PFOA, PFHxS, and PFNA concentrations were measured in 448 stored maternal serum samples collected between 1991 and 1992. PFAS analyses were conducted at the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) using a modification of a previously described analytical method (Kuklenyik, Needham, and Calafat, 2005). The PFOS limit of detection was 0.2 ng/mL and the limit of detection for all other PFAS of interest was 0.1 ng/mL.

Potential Confounders

Potential confounders in the relationship between prenatal PFAS exposure and AMH levels in female offspring were determined *a priori* based on biological plausibility and findings from the literature. The following variables were evaluated as potential confounders: pre-pregnancy body mass index (BMI; kg/m^2), maternal education (categorized as less than ordinary level (O-level), O-level, or greater than O-level), prenatal smoking (any vs. none during last two months of pregnancy), maternal age at delivery (years), gestational age at sample collection (weeks), and breastfed status (ever vs. none). Analyses with categorized maternal age at delivery did not differ compared to those with age treated as a continuous variable of interest.

Analyses

All analyses were conducted using SAS 9.3 (Carey, NC). Descriptive analyses were conducted on the subset of 446 mother daughter dyads with complete exposure information for each PFAS. Two of the original 448 samples had missing information on at least one PFAS of interest and were not included in the sample. Analysis of variance tests were utilized to calculate the p-value comparing mean PFAS value for each level of the covariate. AMH levels were log-transformed for analyses due to a gross violation of normality in the distribution of the outcome. Researchers evaluated confounders through a combination of graphical and change-in-estimate methods. Crude estimates from linear regression models of PFAS exposure and AMH levels were compared to adjusted estimates for each potential confounder. Backwards elimination multiple linear regression

analyses were conducted to identify the best model using mother-daughter dyads with information on PFAS exposure and AMH levels (N=173).

Multiple imputation was applied to a subset of mother-daughter dyads with missing data on covariates of interest and AMH. The imputed dataset provided a total study sample of 446 mother-daughter dyads with no missing data. Previously identified multiple linear regression models were applied to the imputed dataset to arrive at the final estimates for the crude and adjusted association between prenatal PFAS exposure and AMH levels in female offspring.

Results

The majority of mothers in this study were white women with ordinary levels of education or higher, who did not smoke during pregnancy (Table 1). Among mothers in the study population, median PFAS concentrations (ng/mL) were: PFOS 19.8 (IQR:15.1, 24.9), PFOA 3.7 (IQR: 2.8, 4.8), PFHxS 1.6 (IQR: 1.2, 2.2), PFNA 0.5 (IQR: 0.4, 0.7). Median PFOS concentrations among mothers differed by prenatal smoking status and median PFOA concentrations differed by maternal race.

The geometric mean AMH concentration was 3.88 ng/mL (IQR: 2.67, 6.37). The distribution of study population characteristics differed between the mother-daughter dyads with no missing data on AMH and the mother-daughter dyads with missing data on AMH (61.2%). Mothers of daughters with missing AMH were more likely to smoke at least one cigarette during pregnancy and never breastfeed during pregnancy compared to mothers of daughter without missing AMH (Table 2).

Model results of imputed analyses (N=446) were compared to the multivariable linear regression analyses for the complete-case analysis with no missing AMH (N=173; Appendix Table 1). Differences between the two results suggested the complete-case analysis was biased with respect to exclusion of missing data. Due to the discrepancy between the imputed analyses and complete-case analyses, results using the imputed model were reported (Table 3).

In the unadjusted models, mean differences in AMH per one ng/mL higher in PFOS, PFOA PFHxS, and PFNA were 0.58% (CI: -0.3%, 1.4%, p=0.17), 3.1% (CI: 2.2%, 8.4%, p=0.23), 0.9% (CI: -0.5%, 2.3%, p=0.18), 9.5% (CI: -79.6%, 98.6%, p=0.79). The multivariable adjusted and unadjusted models were similar (Table 3). The results of the adjusted models indicate mean differences in AMH per one ng/mL higher PFOS, PFOA, PFHxS, and PFNA were 0.5% (CI: -0.4%, 1.5%, p=0.24), 2.9% (CI: -2.3%, 8.2%, p=0.25), 0.9% (CI: -0.5%, 22.8%, p=0.21), 8.0% (-76.9%, 93.0%, p=0.82). No significant associations were observed between maternal PFAS exposure and AMH levels in daughters. There was no evidence of effect modification by pre-pregnancy BMI, maternal education, maternal age at delivery, or breastfed status.

Discussion

In this study, we observed no associations between a range of prenatal PFAS exposures, including PFOS, PFOA, PFHxS, and PFNA, and AMH, a marker of ovarian reserve, in female offspring. These findings are consistent with the only previous study, to our knowledge, that evaluated the association between maternal PFAS exposure and AMH levels in adolescent girls (n=343). The prior study used a Danish cohort and found

no associations between prenatal PFOS or PFOA exposure and concentrations of AMH in female offspring approximately 20 years old (Kristensen et al, 2013).

While we did not control for race/ethnicity in this analysis due to the small number of non-white girls in the sample, previous work suggests AMH levels do not vary greatly by race/ethnicity (Elchuri et al, 2015). Additionally, we did not control for prenatal smoking in the final analysis as it was not determined to be a confounder. This is in line with an ALSPAC analysis among 1,144 mother daughter-dyads, which found no strong association between maternal smoking and AMH levels in daughters 15 years of age (Fraser et al., 2013).

This analysis contributes to a growing body of literature examining the effects of environmental exposures, specifically PFAS, on human health. Although previous research found no association between PFOA and PFOS and AMH levels in female offspring at 20 years old (n=344), this analysis assessed the effect of two additional PFAS, PFxHS and PFNA, with AMH levels in daughters at 15.5 years of age and utilized a larger sample size (n=446) (Kristensen et al., 2013). This is a valuable contribution as AMH levels deplete over time (Weenen et al., 2004). Researchers utilized a well characterized dataset for all analyses and had access to a wide range of covariates.

Limitations of this study include the use of maternal serum PFAS concentrations as a proxy for prenatal PFAS exposure among female offspring. However, this method of assessing prenatal PFAS exposure is commonly used in multiple studies as PFAS can cross the placental barrier (Kristensen et al, 2013). Further, the small number of non-

white girls in the sample prevented researchers from assessing race/ethnicity as a potential effect modifier in the relationship between PFAS and AMH.

Future Directions

Future studies could utilize a population that includes a greater percentage of non-white girls to assess the relationship between prenatal PFAS exposure and AMH levels in daughters. This type of population would allow for the assessment of race/ethnicity as a potential effect modifier. Additional analyses might also evaluate this relationship at different time points in the daughter's life.

References

Anderson, E. L., Fraser, A., McNally, W., Sattar, N., Lashen, H., Fleming, R., . . . Lawlor, D. A. (2013).Anti-mullerian hormone is not associated with cardiometabolic risk factors in adolescent females. *PloS One, 8*(5), e64510. doi: 10.1371/journal.pone.0064510

Christensen, K. Y., Maisonet, M., Rubin, C., Holmes, A., Calafat, A. M., Kato, K., . . . Marcus, M. (2011). Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. *Environment International, 37*(1), 129-135. doi: 10.1016/j.envint.2010.08.007

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., …Gore, A. C. (2009). Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocrine Reviews*, *30*(4), 293–342. http://doi.org/10.1210/er.2009-0002

Elchuri, S. V., Patterson, B. C., Brown, M. R., Buchanan, I., Mertens, A. C., & Meacham, L. R. (2015). Anti-Mullerian hormone levels in American girls by age and race/ethnicity. *Journal of Pediatric Endocrinology and Metabolism*, *28*(1-2), 189-193. doi: 10.1515/jpem-2014-0242

Hansen, K. R., Hodnett, G. M., Knowlton, N., & Craig, L. B. (2011). Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertility and Sterility, 95*(1), 170-175. doi: http://dx.doi.org/10.1016/j.fertnstert.2010.04.006

Kato, K., Wong, L.-Y., Jia, L. T., Kuklenyik, Z., & Calafat, A. M. (2011). Trends in Exposure to Polyfluoroalkyl Chemicals in the U.S. Population: 1999−2008. *Environmental Science & Technology, 45*(19), 8037-8045. doi: 10.1021/es1043613

Kissa, E. (2001) *Fluorinated Surfactants and Repellants*, 2nd ed. Marcel Decker, New York.

Kristensen, S. L., Ramlau-Hansen, C. H., Ernst, E., Olsen, S. F., Bonde, J. P., Vested, A., . . . Toft, G. (2013). Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. *Human Reproduction, 28*(12), 3337-3348. doi: 10.1093/humrep/det382

Fraser, A., Macdonald-Wallis, C., Tilling, K., Boyd, A., Golding, J., Davey Smith, G., … Lawlor, D. A. (2013). Cohort Profile: The Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *International Journal of Epidemiology*, *42*(1), 97–110. http://doi.org/10.1093/ije/dys066

Fraser, A., McNally, W., Sattar, N., Anderson, E. L., Lashen, H., Fleming, R., . . . Nelson, S. M. (2013). Prenatal exposures and anti-Mullerian hormone in female adolescents: the Avon Longitudinal Study of Parents and Children. *American Journal of Epidemiology, 178*(9), 1414-1423. doi: 10.1093/aje/kwt137

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., & Seed, J. (2007). Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicological Sciences, 99*(2), 366-394. doi: 10.1093/toxsci/kfm128

Needham, L. L., Grandjean, P., Heinzow, B., Jørgensen, P. J., Nielsen, F., Patterson, D. G., … Weihe, P. (2011). Partition of Environmental Chemicals between Maternal and Fetal Blood and Tissues. *Environmental Science & Technology*, *45*(3), 1121–1126. http://doi.org/10.1021/es1019614

te Velde, E. R., & Pearson, P. L. (2002). The variability of female reproductive ageing. *Human Reproduction Update, 8*(2), 141-154.

Tsai, M. S., Lin, C. Y., Lin, C. C., Chen, M. H., Hsu, S. H., Chien, K. L., . . . Su, T. C. (2015). Association between perfluoroalkyl substances and reproductive hormones in adolescents and young adults. *International Journal of Hygiene and Environmental Health, 218*(5), 437-443. doi: 10.1016/j.ijheh.2015.03.008

Vélez, M. P., Arbuckle, T. E., & Fraser, W. D. (2015). Maternal exposure to perfluorinated chemicals and reduced fecundity: the MIREC study. *Human Reproduction (Oxford, England)*, *30*(3), 701–709. http://doi.org/10.1093/humrep/deu350

Visser, J. A., de Jong, F. H., Laven, J. S., & Themmen, A. P. (2006). Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction, 131*(1), 1-9. doi: 10.1530/rep.1.00529

Wallace, A. M., Faye, S. A., Fleming, R., & Nelson, S. M. (2011). A multicentre evaluation of the new Beckman Coulter anti-Müllerian hormone immunoassay (AMH Gen II). *Annals of Clinical Biochemistry, 48*(4), 370-373. doi: 10.1258/acb.2011.010172

Weenen, C., Laven, J. S. E., von Bergh, A. R. M., Cranfield, M., Groome, N. P., Visser, J. A., . . . Themmen, A. P. N. (2004). Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *MHR: Basic science of reproductive medicine, 10*(2), 77-83. doi: 10.1093/molehr/gah015

Zoeller, R. T., Brown, T. R., Doan, L. L., Gore, A. C., Skakkebaek, N. E., Soto, A. M., … Vom Saal, F. S. (2012). Endocrine-Disrupting Chemicals and Public Health

Protection: A Statement of Principles from The Endocrine Society. *Endocrinology*, *153*(9), 4097–4110. http://doi.org/10.1210/en.2012-1422

Table 1: Study population characteristics in a subset of the Avon Longitudinal Study for Parents and Children (N=446 mother-daughter dyads)

	Frequency [n(%)]	PFOS (ng/mL) Median (IQR)	PFOA (ng/mL) Median (IQR)	PFHxS (ng/mL) Median (IQR)	PFNA (ng/mL) Median (IQR)
Overall	446	19.8 (15.1, 24.9)	3.7 (2.8, 4.8)	1.6 (1.2, 2.2)	0.5 (0.4, 0.7)
Maternal pre-pregnancy BMI[a]					
Underweight (<18.5)	18 (4.0)	16.9 (14.0, 22.6)	3.5 (2.8, 4.7)	1.5 (1.2, 2.3)	0.5 (0.3, 0.6)
Normal (18.5-24.9)	289 (64.8)	20.1 (15.1, 25.5)	3.8 (2.8, 4.8)	1.6 (1.2, 2.2)	0.5 (0.4, 0.7)
Overweight (25.0-29.9)	62 (13.9)	20.9 (17.5, 25.6)	3.7 (3.2, 4.9)	1.9 (1.5, 2.5)	0.6 (0.4, 0.7)
Obese (≥30.0)	31 (7.0)	19.2 (13.8, 23.4)	3.6 (2.7, 5.0)	1.4 (1.2, 2.3)	0.6 (0.3, 0.7)
Missing	46 (10.3)	17.3 (13.4, 22.4)	3.5 (2.8, 4.5)	1.6 (1.3, 2.3)	0.5 (0.3, 0.7)
Prenatal smoking					
Any	79 (17.7)	17.2 (13.4, 21.4)*	3.4 (2.9, 4.4)	1.7 (1.3, 2.4)	0.5 (0.3, 0.7)
None	346 (77.6)	20.6 (15.4, 25.6)*	3.8 (2.8, 4.9)	1.6 (1.2, 2.2)	0.6 (0.4, 0.7)
Missing	21 (4.7)	20.8 (15.6, 22.9)*	4.0 (3.2, 4.4)	1.6 (1.2, 1.9)	0.5 (0.4, 0.7)
Maternal race					
White	422 (94.6)	19.9 (15.2, 25.3)	3.8 (2.9, 4.8)*	1.6 (1.2, 2.2)	0.5 (0.4, 0.7)
Non-white	7 (1.6)	14.6 (11.8, 19.2)	2.4 (2.0, 2.9)*	1.4 (1.0, 1.7)	0.5 (0.2, 0.7)
Missing	17 (3.8)	18.5 (12.7, 22.4)	3.3 (2.5, 4.1)*	1.6 (1.1, 2.3)	0.5 (0.4, 0.7)
Maternal age at delivery[b]					
<25	92 (20.6)	18.5 (14.1, 23.1)	3.9 (3.0, 4.8)	1.6 (1.2, 2.1)	0.5 (0.4, 0.6)
25-29	164 (36.8)	20.7 (15.4, 25.4)	3.8 (3.0, 4.9)	1.6 (1.2, 2.1)	0.6 (0.4, 0.7)
>29	187 (41.9)	19.7 (15.1, 25.6)	3.6 (2.5, 4.6)	1.7 (1.2, 2.4)	0.5 (0.4, 0.7)
Missing	3 (0.7)	22.4 (17.0, 22.7)	2.8 (2.2, 4.4)	1.6 (0.5, 3.7)	0.6 (0.4, 0.7)
Maternal education[c]					
Less than O-level	89 (20.0)	18.2 (14.9, 23.3)	3.6 (2.8, 4.4)	1.6 (1.3, 2.2)	0.5 (0.4, 0.7)
O-level	140 (31.4)	19.6 (15.1, 26.0)	3.7 (2.9, 5.0)	1.6 (1.2, 2.3)	0.6 (0.4, 0.7)
Greater than O-level	198 (44.4)	20.4 (15.2, 25.3)	3.9 (2.8, 4.8)	1.7 (1.2, 2.2)	0.5 (0.4, 0.7)
Missing	19 (4.3)	17.0 (13.7, 22.7)	3.3 (2.5, 4.4)	1.6 (1.1, 2.3)	0.5 (0.4, 0.7)
Breastfed status					
Never	82 (18.4)	19.8 (15.3, 25.5)	4.1 (3.1, 5.1)	1.6 (1.3, 2.3)	0.5 (0.4, 0.7)
Ever	332 (74.4)	19.8 (15.0, 24.8)	3.7 (2.8, 4.8)	1.6 (1.2, 2.2)	0.5 (0.4, 0.7)
Missing	32 (7.2)	18.6 (14.4, 23.4)	3.5 (2.6, 4.4)	1.7 (1.2, 2.4)	0.6 (0.4, 0.7)

[a] Maternal pre-pregnancy BMI, measured in kg/m^2

[b]Maternal age at delivery, measured in years

[c] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Table 2: Distribution of study population characteristics comparing individuals with missing AMH to individuals without missing AMH (N=446)

	No Missing AMH Frequency [n(%)]	Missing AMH Frequency [n(%)]	p-value
Overall	173	273	
Maternal pre-pregnancy BMI, kg/m^2			
Underweight (<18.5)	8 (4.6)	10 (3.7)	0.275
Normal (18.5-24.9)	113 (65.3)	176 (64.5)	
Overweight (25.0-29.9)	26 (15.0)	36 (13.2)	
Obese (≥30.0)	8 (4.6)	23 (8.4)	
Missing	18 (10.4)	28 (8.4)	
Prenatal smoking			
Any	21 (12.1)	58 (21.3)	0.010
None	146 (84.4)	200 (73.3)	
Missing	6 (3.5)	15 (5.5)	
Maternal race			
White	164 (94.8)	258 (94.5)	0.327
Non-white	4 (2.3)	3 (1.1)	
Missing	5 (2.9)	12 (4.4)	
Maternal age at delivery, years			
<25	23 (13.3)	69 (25.3)	0.002
25-29	65 (37.6)	99 (36.3)	
>29	84 (48.6)	103 (37.7)	
Missing	1 (0.6)	2 (0.7)	
Maternal education[a]			
Less than O-level	26 (15.0)	63 (23.1)	0.003
O-level	50 (28.9)	90 (33.0)	
Greater than O-level	92 (53.2)	106 (38.8)	
Missing	5 (2.9)	14 (5.1)	
Breastfed status			
Never	23 (13.3)	59 (21.6)	0.025
Ever	138 (79.8)	194 (71.1)	
Missing	12 (6.9)	20 (7.3)	

[a] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Table 3: Crude and Adjusted* Models using Multiple Imputation (N=446)

	Model	Beta	95% CI	p-value
PFOS	Crude	0.0058	(-0.003, 0.014)	0.17
	Adjusted	0.0055	(-0.004, 0.015)	0.24
PFOA	Crude	0.0312	(-0.022, 0.084)	0.23
	Adjusted	0.0292	(-0.023, 0.082)	0.25
PFHxS	Crude	0.0093	(-0.005, 0.023)	0.18
	Adjusted	0.0087	(-0.005, 0.228)	0.21
PFNA	Crude	0.0951	(-0.796, 0.986)	0.79
	Adjusted	0.0801	(-0.769, 0.929)	0.82

*adjusted for maternal age at delivery (years), pre-pregnancy BMI (kg/m^2), and maternal education (< O-level, O-level, > O-level)

Appendix Table 1: Crude and Adjusted Models without missing AMH (N=173)

		Beta	95% CI	p-value
PFOS	Crude	0.0081	(0.0017, 0.0145)	0.01
	Adjusted	0.0120	(0.0039, 0.0202)	0.004
PFOA	Crude	0.0376	(0.0042, 0.0709)	0.03
	Adjusted	0.0496	(0.0083, 0.0908)	0.02
PFHxS	Crude	0.0083	(-0.0006, 0.0172)	0.07
	Adjusted	0.0129	(0.0020, 0.0239)	0.02
PFNA	Crude	0.0941	(-0.2834, 0.4717)	0.63
	Adjusted	0.0465	(-0.3909, 0.4840)	0.84

*adjusted for maternal age at delivery (years), pre-pregnancy BMI (kg/m^2), and maternal education (< O-level, O-level, > O-level)