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Perfluoroalkyl Chemicals, Menstrual Cycle Length, and Fecundity: Findings from a Prospective Pregnancy Study

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Abstract

Background—Perfluoroalkyl substances have been associated with changes in menstrual cycle characteristics and fecundity, when modeled separately. However, these outcomes are biologically related, and we evaluate their joint association with exposure to perfluoroalkyl substances.

Methods—We recruited 501 couples from Michigan and Texas in 2005-2009 upon their discontinuing contraception and followed them until pregnancy or 12 months of trying. Female partners provided a serum sample upon enrollment and completed daily journals on menstruation, intercourse, and pregnancy test results. We measured seven perfluoroalkyl substances in serum using liquid-chromatography-tandem mass spectrometry. We assessed the association between perfluoroalkyl substances and menstrual cycle length using accelerated failure time models and between perfluoroalkyl substances and fecundity using a Bayesian joint modeling approach to incorporate cycle length.

Results—Menstrual cycles were 3% longer comparing women in the second versus first tertile of perfluorodecanoate (PFDeA; acceleration factor [AF]=1.03, 95% credible interval [CrI]=[1.00, 1.05]), but 2% shorter for women in the highest versus lowest tertile of perfluorooctanoic acid (PFOA) (AF=0.98, 95% CrI=[0.96, 1.00]). When accounting for cycle length, relevant covariates and remaining perfluoroalkyl substances, the probability of pregnancy was lower for women in second versus first tertile of PFNA (odds ratio [OR]=0.6, 95% CrI=[0.4, 1.0]) though not when comparing the highest versus lowest (OR=0.7, 95% CrI=[0.3, 1.1]) tertile.

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List of Supplemental Digital Content

- Supplemental Digital Content. eAppendix A describes model specification and assumptions; eTables 1 and 2, eFigure 1. pdf

Conclusions—In this prospective cohort study, we observed associations between two perfluoroalkyl substances and menstrual cycle length changes, and between select perfluoroalkyl substances and diminished fecundity at some (but not all) concentrations.

INTRODUCTION

Perfluoroalkyl substances, formerly referred to as perfluorochemicals (PFCs), are a group of persistent synthetic chemicals with a variety of commercial uses (e.g., surfactants and surface protectors in carpets, leather, paper, packaging, fabric and upholstery),¹ which likely accounts for their detection in human biomonitoring initiatives.²⁻⁴ The primary source of human exposure to perfluoroalkyl substances is consumption of contaminated food,^{5,6} with one study estimating a daily dose of 2-3 ng/kg.⁷ Other exposure pathways include indoor and outdoor air, household dust, and to a lesser extent placental transfer.⁷ Perfluorooctane sulfonate (PFOS) has been listed as a persistent organic pollutant,⁸ given its long half-life and environmental persistence. Unlike some other persistent organic pollutants, perfluoroalkyl substances are not lipophilic; rather, they bind to serum albumin allowing for their quantification in serum or plasma.⁹ Over 60 years since the introduction of perfluoroalkyl substances, scientists from 38 countries have signed the Madrid Statement calling for global actions to restrict usage of these chemicals and develop alternatives using nonfluorinated chemicals.¹⁰

Research on the potential toxicity of perfluoroalkyl substances to women's reproductive health is emerging. Recent studies indicate that serum perfluorooctanoic acid (PFOA) and perfluorononanoate (PFNA) may be associated with increased odds of endometriosis,¹¹ serum PFNA and perfluorodecanoate (PFDeA) with increased odds of miscarriage,¹² serum PFOA and perfluoroundecanoate (PFUA) with altered reproductive hormonal profiles in female adolescents and young adults,¹³ which may have implications for menstrual cycles and fecundity. A cross-sectional study of pregnant women reported that women in the highest tertile of serum PFOA concentration versus the lowest were more likely to have longer menstrual cycles (32 days), based upon retrospectively reported menses data.¹⁴ Additionally, women in the third versus first tertile of PFOS concentration tended to have more irregular cycles,¹⁴ supporting findings based upon pregnant women in the Danish National Birth Cohort retrospectively reporting menses. Specifically, women with plasma concentrations 25th percentiles for PFOA and PFOS were more likely to report irregular cycles than women with the lowest concentrations.¹⁵

With regard to female fecundity (the biologic capacity of women for reproduction),¹⁶ as measured by either the observed or retrospectively reported number of months/cycles needed to become pregnant, increasing concentrations of PFOA, PFOS, and perfluorohexane sulfonate (PFHxS) have been associated with reduced human fecundity or a longer time-to-pregnancy (TTP) in studies of pregnant women.^{15,17-20} In a prospective study of couples attempting pregnancy, increasing serum perfluorooctane sulfonamide (PFOSA) (log transformed and scaled by standard deviation) was associated with a longer TTP.²¹ Yet, data from one prospective²² and one retrospective²³ pregnancy based study did not support associations between perfluoroalkyl substances and TTP. Only one study adjusted TTP for menstrual cycle length,²² despite its biologic relevance for fecundity.²⁴⁻²⁷

Important methodologic issues underlie some of the available evidence on perfluoroalkyl substances, menstrual cycle length, and fecundity. First, studies comprising pregnant women exclude women who are unable to become pregnant and who may have higher perfluoroalkyl substance exposure or extreme cycle lengths. Second, studies assessing the validity of retrospectively reported cycle length have found considerable measurement error²⁸ and a tendency to overestimate time,²⁹ while bi-directional reporting errors have been observed for retrospectively reported TTP relative to prospective measurement.³⁰ Motivated by these findings, we used data from a prospective cohort of couples attempting pregnancy to assess preconception serum concentrations of perfluoroalkyl substances, their relation with cycle length, and also with fecundity, in joint models that account for cycle length and relevant confounders. We examined seven perfluoroalkyl substances including PFNA and PFDeA, for which limited data with respect to female fecundity exist.

METHODS

Study Design and Cohort

We analyzed data from the female partners of couples participating in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. Briefly, this is a prospective cohort study comprising 501 couples residing in 16 counties in Michigan and Texas who were recruited between 2005-2009 upon discontinuing contraception for purposes of becoming pregnant, as fully described elsewhere.³¹ The couples were followed daily until pregnancy (indicated by human chorionic gonadotropin (hCG) concentration ≥ 25 mIU/mL) or 12 months of trying. Eligibility criteria included: in a committed relationship, females aged 18-40 and males 18+ years, English or Spanish speaking, no use of hormonal birth control injections in the past 12 months, off contraception for ≥ 2 months, and self-reported cycle lengths between 21-42 days to comply with fertility monitor requirements. Couples were excluded if either partner reported clinically diagnosed infertility. Full human subjects' approval was obtained from all participating institutions, and all participants provided written informed consent prior to enrollment into the study.

Data and Biospecimen Collection

At enrollment, the research team went to homes to conduct the baseline interviews and perform standardized anthropometric assessments.³² Pregnancy tests were administered to women to ensure they were not already pregnant. Using phlebotomy equipment deemed appropriate for measuring environmental chemicals, non-fasting blood samples (~20mL) were obtained for measurement of perfluoroalkyl substances and cotinine, a biomarker of nicotine for defining active smoking status. Women were trained in the completion of daily journals to capture menses, sexual intercourse and pregnancy test results.

For the duration of the study, women recorded menstrual bleeding intensity in a daily journal. Cycle length was defined as the time (days) from the onset of bleeding (day 1) that increased in intensity and lasted for 2+ days, to the onset of bleeding in the next cycle, supplemented with information from the fertility monitor as to the timing the menstruation onset button was pressed to indicate onset of bleeding. As enrollment occurred on various days of women's menstrual cycles, the length of the first cycle under study was the sum of

the prospectively observed portion (median=15, interquartile range (IQR)=[7, 22] days) and the time since last menstrual period (reported at enrollment). The length of a cycle in which the couple became pregnant, including the enrollment cycle, was censored at day of ovulation and included in the analysis. Women without observed pregnancies either due to withdrawal from the study or unsuccessfully trying for 12 months were censored on the last day of the last fully observed menstrual cycle.

Ovulation was determined from the Clearblue® Easy home fertility monitor, a urinary dipstick device that measures estrone-3-glucuronide and luteinizing hormone (LH). The monitor displays “peak” fertility on the day(s) that LH exceeds a threshold (typically two days), and its accuracy is high for detecting the LH peak (99%) and peak fertility (91%) compared to transvaginal ultrasonography, the gold standard for ovulation.³³ We estimated day of ovulation as the day of peak fertility or the latter of consecutive peak days. If a peak was not detected and the woman tested <90% of the required days, we imputed day of ovulation using the mean of her previously observed cycles (274 cycles, 13%). If no previous cycles were available (41 cycles, 2%), we used the day with the highest LH measurement to define ovulation day.

Intercourse, recorded in the female’s journal, was used to estimate the timing of intercourse relative to day of ovulation. If blank (2% of days), we used the male partner’s report of intercourse, or if blank for both partners (6% of days), we assumed that intercourse did not occur.

We measured fecundity by the probability of pregnancy in a menstrual cycle. Women were trained in the use of Clearblue® Easy digital home pregnancy tests with testing on the day of expected menses. The accuracy of the pregnancy test as used by women is high compared to laboratory detection.³⁴

The 501 enrolled women contributed 2,249 menstrual cycles. However, 16 cycles (<1%) were excluded for insufficient data to establish a cycle, while 25 (1%) cycles were excluded as they were estimated to be <9 days or >89 days in length and 34 (2%) had no ovulation detected or were noncompliant relative to the fertility monitor. Consequently, 2,174 cycles (97%) contributed by 483 women (96%) were available for analysis.

Toxicological Analysis

Quantification of preconception serum concentrations of perfluoroalkyl substances was performed by the Division of Laboratory Sciences at the National Center for Environmental Health, Centers for Disease Control and Prevention (Atlanta, GA), using isotope dilution high-performance liquid-chromatography-tandem mass spectrometry and established operating procedures.^{3,35} Quality assurance and control procedures were followed (e.g., the analysis of calibration standards, blanks, and quality control materials in each batch) to maintain the accuracy and reliability of the measurements. Detailed method performance data are provided elsewhere.³⁵ Seven perfluoroalkyl substances were quantified (ng/mL): perfluorodecanoate (PFDeA), perfluorononanoate (PFNA), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and its precursors, perfluorooctane sulfonamide (PFOSA), 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Et-PFOSA-AcOH) and 2-(N-methyl-

perfluorooctane sulfonamido) acetate (Me-PFOA-AcOH). The limit of detection was 0.1 for PFNA, PFOSA, and PFOA and 0.2 for the remaining perfluoroalkyl substances. In addition, serum cotinine was quantified (ng/mL) using liquid chromatography-isotope dilution tandem mass spectrometry.³⁶ Cotinine concentrations ≥ 10 ng/mL defined active smoking.³⁷

Statistical Analysis

We estimated the distributions of baseline covariates and percentiles of perfluoroalkyl substances (25th, 50th, and 75th) stratified by mean cycle length (24, 25-31, 32 days).^{14,28} In the model-based analyses, we modeled cycle length as a continuous measurement (days) and pregnancy per cycle as binary (yes/no). We categorized most serum perfluoroalkyl substance concentrations *a priori* into tertiles using the lowest as the reference group. However, PFOSA and Et-PFOA-AcOH concentrations were dichotomized as above/below the limit of detection and above/below the 75th percentile, respectively, given the high percentage of measurements less than or equal to the limit of detection.

Our analyses consisted of two linked models (cycle length and probability of pregnancy) fitted using the Bayesian joint modeling approach and a less complex two-stage approach (sensitivity analysis), as described elsewhere²⁷ (see eAppendix A, Supplemental Digital Content). Briefly for the i^{th} ($i=1, \dots, n$) woman, we denote the j^{th} ($j=1, \dots, n_j$) cycle length by Y_{ij} . Covariate effects operate multiplicatively via a hierarchical accelerated failure time model³⁸

$$\left[Y_{ij} | \mathbf{v}_i^T \boldsymbol{\eta}, \mathbf{W}_i, \epsilon_{ij} \right] = e^{(\mathbf{v}_i^T \boldsymbol{\eta})} \times W_i \times \epsilon_{ij}$$

where \mathbf{v}_i denotes covariates including perfluoroalkyl substance group and potential confounders, $\boldsymbol{\eta}$ denotes corresponding regression coefficients, W_i is a woman-specific random effect that accounts for heterogeneity, and ϵ_{ij} is the error term. We adjusted for *a priori* defined potential confounders, age (continuous), body mass index (BMI, <18.5 (underweight), 18.5-24.9 (normal), 25-29.9 (overweight), ≥ 30 kg/m² (obese)), and active smoking based on serum cotinine (<10, ≥ 10 ng/mL) at enrollment. The acceleration factor ($AF=e^{\boldsymbol{\eta}}$), represents the relative change in cycle length for women with higher versus lowest serum perfluoroalkyl substance concentrations. For example, an $AF=0.95$ is interpreted as a decrease in length by 5%. An $AF>1$ corresponds to a longer cycle length for women with higher versus lowest concentrations. We accommodated length-bias sampling for the cycle in which the couple enrolls and right censoring of length for the cycle in which the woman becomes pregnant.^{27,39}

Data for the pregnancy model consisted of a cycle specific pregnancy indicator, denoted A_{ij} , with $A_{ij} \equiv 0, j < n_j$ (i.e. not pregnant in previous cycles), an intercourse occurrence indicator for the k^{th} ($k=1, \dots, Y_{ij}$) day within the j^{th} cycle, denoted x_{ijk} , and the difference in days from the intercourse day to the ovulation day, denoted by d_{ijk} . Covariates denoted \mathbf{z}_i include perfluoroalkyl substance concentration and potential confounders and enter via the hierarchical model,

$$\begin{aligned}
 & Pr(A_{ij}=1 \mid \text{not pregnant in previous cycles}, Y_i^*) \\
 &= 1 - \prod_{l=1}^{Y_{ij}} \{1 - \rho_{ijl}(Y_i^*, \mathbf{z}_i, d_{ijl})\}^{x_{ijl}}, \\
 & \text{logit} \{ \rho_{ijk}(Y_i^*, \mathbf{z}_i, d_{ijk}) \} = \mathbf{z}_i^T \boldsymbol{\gamma} + \beta_1 Y_i^* + \beta_2 (Y_i^*)^2 + \mathbf{g}(d_{ijk});
 \end{aligned}$$

where ρ_{ijk} is the probability of pregnancy by intercourse on the k^{th} day of the cycle and is conditional on intercourse occurring on day k and previous intercourse acts not resulting in pregnancy, Y_i^* is the woman's typical cycle length (latent) obtained from the cycle length model, $\mathbf{g}(\cdot)$ is a flexible spline function which accounts for timing of intercourse relative to the ovulation day. In sensitivity analyses, we considered an extension of this model with a second random effect (see eAppendix A.3 and eTable 1, Supplemental Digital Content).

We estimated the associations between perfluoroalkyl substances and the probability of pregnancy by the odds ratio (OR= e^{γ}). An OR<1 indicates a reduced probability of pregnancy for women in the higher groupings of perfluoroalkyl substance concentration relative to the lowest. In addition to intercourse and cycle length, we adjusted for the pre-specified potential confounders, age (continuous), BMI (<18.5 (underweight), 18.5-24.9 (normal), 25-29.9 (overweight), ≥ 30 kg/m²(obese)), and active smoking based on serum cotinine (<10, ≥ 10 ng/mL) at enrollment. In sensitivity analyses, we added adjustment for parity conditional on gravidity (never pregnant, pregnant without births, pregnant with births). The association between the woman's typical cycle length and the probability of pregnancy is quantified in the log-linear model using both linear and squared terms.

We also modeled perfluoroalkyl substances using the instrument-measured concentrations (i.e., values output from the instrument) irrespective of the limit of detection in keeping with recent practices suggesting substituted values bias the estimation of human health effects.⁴⁰ We scaled the concentrations by their IQR with the exception of PFOSA and Et-PFOSA-AcOH, given their 25th and 75th percentiles were less than or equal to the limit of detection. Additionally, we estimated models using the log transformations of the instrument-measured concentrations without scaling but these analyses did not suggest any associations (results not shown).

We used models that are valid when data are missing at random to impute missing concentrations of perfluoroalkyl substances and to impute cotinine (<4%) concentrations stemming from insufficient serum for quantification. For both the cycle length and pregnancy probability models, we first assessed each perfluoroalkyl substance relative to the outcomes (single perfluoroalkyl substance (PFAS) model), and then included all perfluoroalkyl substances to adjust simultaneously for other perfluoroalkyl substances (multiple perfluoroalkyl substance model). We did not adjust for multiple comparisons, consistent with our interest in exploring all relations between the various perfluoroalkyl substances and our study outcomes in light of considerable data gaps.

Imputations and the posterior distributions of the parameters and functions of interest were generated using Markov Chain Monte Carlo (MCMC) methods⁴¹ implemented in

OpenBUGS v3.2.3 (Helsinki, Finland). Posterior distributions are summarized by the median and 95% credible interval (CrI), which are Bayesian analogues of the frequentist point estimate and confidence interval. If the model is correctly specified, the meaning of the 95% credible interval is that there is a 95% probability that the unknown value of the parameter is in this interval based on the data and prior assumptions.⁴¹

RESULTS

Characteristics of the women by mean cycle length are shown in Table 1. The majority of the women in the cohort were Non-Hispanic White, college-educated, with health insurance and non-smokers. Mean cycle length was shorter with increasing age, higher BMI and active smoking status.

The distribution of serum concentrations (ng/mL) of each perfluoroalkyl substance is described in Table 2 stratified by mean cycle length. Essentially all women had detectable concentrations of PFOS and PFOA above or equal to the limit of detection, whereas most women were below the limit of detection for Et-PFOA-AcOH and PFOA, i.e., 97% and 89%, respectively. Women with mean cycle length of 25-31 days had higher levels of PFOA concentrations relative to women with longer mean cycle length. No clear pattern emerged for distributions of perfluoroalkyl substances and mean cycle length. The correlations between the concentrations of perfluoroalkyl substances were mostly below 0.5 with only that for PFDeA and PFNA exceeding 0.7 (eTable 2, Supplemental Digital Content).

Associations between Female Perfluoroalkyl Substances and Cycle Length

Table 3 shows that the direction of the relation between each perfluoroalkyl substance and cycle length was positive for some substances and negative for others. When modeled individually, PFDeA was associated with a 3% increase (AF=1.03, 95% CrI=[1.00, 1.05]) in cycle length (approximately 1 day longer) when comparing women in the second versus first tertile of PFDeA concentration, as was the finding for PFNA (1.02, [0.99,1.04]) in results both unadjusted and adjusted for age, BMI, and active smoking. Similar adjusted results were seen for PFDeA and PFNA when comparing women in the third versus first tertile. Conversely, higher concentrations of PFOA were associated with a 2% reduction (second vs first tertile: 0.98 [0.95, 1.01], third vs first tertile: 0.98 [0.96, 1.00]) in cycle length (Table 3 and eFigure 1, Supplemental Digital Content), representing a decrease of approximately 1 day. A decrease of similar magnitude was observed for Me-PFOA-AcOH comparing women in the second versus first tertile, but not third versus first.

In the models inclusive of all perfluoroalkyl substances, the magnitude and direction of the estimates were consistent with those from the single perfluoroalkyl substance models, though some CrIs were slightly wider. When adjusted for the other perfluoroalkyl substances, we observed a stronger negative association for PFOA; specifically, cycle length was decreased by 5% (approximately 1.5 days) (second vs first tertile: 0.95 [0.93, 0.99], third vs first tertile: 0.95 [0.92, 0.98]).

Association between Cycle Length and Probability of Pregnancy

When estimating pregnancy probability, we included both a linear and quadratic term for cycle length to allow for the possibility of a curvilinear relation. The estimated ORs corresponding to the linear and quadratic terms (Table 4) suggest an inverted U-shaped curve in which extremely short and long cycle lengths are negatively associated with the probability of pregnancy as depicted previously (see Figure 2c).²⁷

Associations between Perfluoroalkyl Substances and Probability of Pregnancy, Adjusted for Cycle Length

Select perfluoroalkyl substances were negatively associated with the probability of pregnancy as indicated by an $OR < 1$ in the model for the day-specific probability of pregnancy (Table 4). Specifically, comparing women in the third versus first tertile of PFOA concentration, we observed diminished probability of pregnancy ($OR = 0.7$ [0.5, 1.0]) when adjusting for intercourse and cycle length. This association was slightly attenuated (0.7 [0.5, 1.1]) with further adjustment for age, BMI and active smoking at enrollment. A similar OR was observed for PFDeA (0.7 , [0.5, 1.1]) comparing the second vs first tertiles, but not the third vs first (0.9 , [0.6, 1.3]). For the model inclusive of all perfluoroalkyl substances, we observed a negative association between PFNA and the probability of pregnancy (Adjusted $OR = 0.6$ [0.4, 1.0]) when comparing women in the second versus first tertile, though not for the third versus first (0.7 [0.3, 1.1]) tertiles. Lastly, when modeling instrument-measured values of perfluoroalkyl substances, we observed consistent ORs though each CrI included 1.

In each of the sensitivity analyses, the direction and magnitude of the point estimates were largely consistent with the primary findings. In particular, we again observed a negative association between PFNA and the probability of pregnancy when comparing women in the second vs first tertile in multiple perfluoroalkyl substance models with a second random effect (0.6 [0.3, 1.0]), with adjustment for parity (0.6 [0.3, 0.9]), and when using a two stage estimation approach (0.6 [0.4, 1.0]). Further, in all three sensitivity analyses, we additionally observed diminished probability of pregnancy comparing women with PFOSA serum concentration above vs below the limit of detection in both single and multiple perfluoroalkyl substance models. Finally, point estimates remained below 1 comparing women in the second vs first tertile of PFDeA concentration but not the third vs first.

DISCUSSION

We found that specific perfluoroalkyl substances at environmentally relevant concentrations were associated with both prospectively observed cycle length and the probability of pregnancy, suggestive of diminished female fecundity. Women in the second and third tertile of serum PFOA had shorter (5%) cycles compared to women in the lowest tertile. Conversely, women in the second versus lowest tertile of serum PFDeA had longer (3%) cycles. Assuming an average cycle length of 29 days, these findings reflect changes of approximately -1.5 and 1 day, respectively. Changes in either direction may be of consequence as we observed both shorter and longer cycle lengths to be associated with

lower pregnancy probabilities in comparison to average cycle length corroborating earlier reports.²⁴⁻²⁷

With regard to perfluoroalkyl substances and the probability of pregnancy, we used a joint modeling approach to account for cycle length and found some evidence suggesting select serum concentrations of PFNA (second vs first tertile) and PFOA (third vs first tertile) to be associated with a lower probability of pregnancy. The observation for PFNA is concerning in light of increasing concentrations of this chemical published in biomonitoring reports for U.S. women, with approximately a 130% increase in median serum PFNA concentration between 1999-2000 and 2009-2010.⁴ In three analyses of sensitivity to model specification, we obtained consistent results and additionally identified a negative association between PFOSA and the probability of pregnancy. Collectively, these findings suggest that exposure to specific perfluoroalkyl substances at some but not all concentrations may be associated with diminished female fecundity as reflected in alterations in cycle length and lower pregnancy probabilities.

An important contribution of this work is the addition of evidence using prospective capture of cycle length, daily intercourse, cycle ovulation day, and time-to-pregnancy. Our findings are strengthened by reliable laboratory quantification of the perfluoroalkyl substances, relatively complete follow-up over a long period of observation, and the ability to quantify preconception exposures. Also, our joint modeling approach incorporates uncertainty in cycle length by repeatedly sampling from the entire posterior distribution of cycle length. The Bayesian toolbox of Markov chain Monte Carlo algorithms offers a straightforward, feasible manner for fitting joint models containing non-linear relations and for obtaining straightforward interpretable interval estimates with complete accounting for all uncertainties.

The limited number of previous investigations on perfluoroalkyl substances and female fecundity makes it challenging to more fully interpret our findings. To our knowledge, menstrual cycle characteristics have not been previously assessed in relation to PFDeA. With regard to PFOA, we observed approximately a 2%-5% reduction in cycle length associated with higher concentrations of PFOA, while a previous study reported an 80% increase in the odds of longer (≥ 32 days) mean cycle length.¹⁴ One explanation is that the former study prospectively captured menses while the latter asked women to recall menses, which is reported to be susceptible to measurement error²⁸ and overestimation.²⁹ Another explanation is that women in the LIFE Study had higher median PFOA concentrations than women participating in the latter or INUENDO Study (3.2 ng/mL vs. 1.5 ng/mL, respectively). Yet another explanation is the sampling framework with the LIFE Study relying on preconception enrollment of women irrespective of their ability to become pregnant, while the latter study enrolled pregnant women.

Some of our findings between perfluoroalkyl substances and fecundity have been reported in earlier studies while others add to the growing evidence for the association of perfluoroalkyl substances with adverse health outcomes. Specifically, our finding from the sensitivity analyses that PFOSA was associated with a lower day-specific probability of pregnancy supports the previous finding from the LIFE Study of a lower fecundability odds ratio for

PFOSA.²¹ Adverse associations between PFOA and fecundity as observed here for women in the third tertile have also been noted in earlier studies,^{15,17-19} including the recently reported MIREC Study.²⁰ In contrast, we know of only three studies of fecundity and either PFDeA or PFNA,²¹⁻²³ which came into production later and have not been extensively studied. Our finding of a negative association between PFNA and fecundity, comparing women in the second vs first tertile, supports the lower fecundability odds ratio reported for higher versus lower concentrations of PFNA in one of these three studies; however, this study also reported inconsistent results restricting to primiparous women.²³ We did not stratify by parity in the primary analysis because it is not a confounder, as the pathway goes from fecundity to parity (not the reverse).⁴² Also, trans-placental and trans-lactational transfer from mother to infant are lower in comparison to lipophilic chemicals.⁷ At the reviewers' request, we added adjustment for parity conditional on gravidity and observed point estimates consistent with our primary findings.

Our findings require careful interpretation for several reasons. One is the utilization of commercially available products for measuring ovulation and pregnancy rather than the gold standard of transvaginal ultrasonography.³³ Also, we did not include male partners' serum perfluoroalkyl substance concentrations in light of our specific focus on menses and pregnancy. Given that pregnancy is a couple dependent outcome, we cannot rule out that the relevant exposure may be that of the male rather than the female partner. Further, caution is advised in interpreting the association between higher PFOSA and diminished fecundity since only 11% of PFOSA concentrations were above the limit of detection, potentially reflective of their ceased U.S. production in 2002 (3M Company 2000).

In conclusion, we observed that female preconception serum levels of PFOA and PFDeA were associated with changes in cycle length. Additionally, we found short and long cycle length were associated with diminished fecundity as measured by lower probabilities of pregnancy. Using joint and two-stage approaches, we adjusted for cycle length in pregnancy models and found some evidence that PFNA, PFOA, PFDeA and PFOSA were associated with a lower probability of pregnancy in some but not all comparisons. Our findings await corroboration as investigation in the role of perfluoroalkyl substances, in particular the lesser studied PFNA and PFDeA, and female cycle length along with fecundity has only recently begun.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline Characteristics by Mean Menstrual Cycle Length, LIFE Study, 2005-2009.

Baseline Characteristic	24 Day Cycle (n=21)	25-31 Day Cycle (n=305)	32 Day Cycle (n=157)
Age (years), Mean \pm SD	32.1 \pm 4.8	30.5 \pm 4.2	29.0 \pm 3.6
BMI (kg/m ²), Mean \pm SD	28.3 \pm 9.3	27.3 \pm 6.5	27.9 \pm 8.2
Active smoking ^a , No. (%)	3 (14)	29 (10)	22 (14)
Gravid, No. (%)	12 (57)	186 (61)	79 (50)
Parous, No. (%)	12 (57)	153 (51)	61 (39)
Nonwhite, No. (%)	1 (5)	42 (14)	24 (15)
High school graduate/GED, No. (%)	0 (0)	16 (5)	9 (6)
No health insurance, No. (%)	2 (10)	17 (6)	17 (11)

GED indicates general education development (equivalent to high school diploma).

^aDefined as serum cotinine \geq 10ng/mL.

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Percentiles of Serum PFAS Concentrations (ng/mL) by Mean Menstrual Cycle Length, LIFE Study, 2005-2009.

Table 2

PFAS	<LOD ^a (%)	24 Day Cycle (n=21)			25-31 Day Cycle (n=305)			32 Day Cycle (n=157)		
		25 th	50 th	75 th	25 th	50 th	75 th	25 th	50 th	75 th
PFAS										
PFOSA	89	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Et-PFOSA-AcOH	97	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1
Me-PFOSA-AcOH	25	0.2	0.4	0.7	0.1	0.3	0.5	0.2	0.3	0.5
PFDeA	9	0.2	0.4	0.5	0.2	0.4	0.6	0.2	0.4	0.5
PFNA	1	0.7	1.3	1.5	0.8	1.2	1.7	0.7	1.1	1.7
PFOS	<1	9.7	12.3	17.0	8.2	12.6	17.6	7.3	11.5	16.9
PFOA	<1	2.5	3.1	4.0	2.3	3.5	5.0	2.0	3.1	4.7

Et-PFOSA-AcOH indicates 2-(N-ethyl-perfluorooctane sulfonamido) acetate; LOD, limit of detection; Me-PFOSA-AcOH, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, perfluoroalkyl substance; PFDeA, perfluorodecanoate; PFNA, perfluorononanoate; PFOSA, perfluorooctane sulfonamide; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate.

^aLOD was 0.1 ng/mL for PFNA, PFOSA, and PFOA, and 0.2 ng/mL for Et-PFOSA-AcOH, Me-PFOSA-AcOH, PFDeA, and PFOS.

Table 3

Unadjusted and Adjusted Associations Between Serum PFAS and Menstrual Cycle Length Estimated Using Bayesian Joint Model, LIFE Study, 2005-2009.

PFAS (ng/mL)	Single PFAS Model ^a		Multiple PFAS Model ^b	
	Unadjusted AF (95% CrI)	Adjusted ^c AF (95% CrI)	Adjusted ^d AF (95% CrI)	Adjusted ^e AF (95% CrI)
PFOSA				
LOD (0.10)	0.99 (0.96, 1.02)	0.99 (0.96, 1.02)	0.99 (0.95, 1.02)	0.98 (0.95, 1.02)
Et-PFOSA-AcOH				
Q3 (0.06)	1.00 (0.97, 1.02)	1.01 (0.99, 1.04)	1.00 (0.97, 1.03)	1.01 (0.98, 1.04)
Me-PFOSA-AcOH				
Tertile 2 (0.02-0.03)	0.97 (0.94, 0.99)	0.98 (0.95, 1.01)	0.97 (0.94, 1.00)	0.98 (0.95, 1.00)
Tertile 3 (0.04)	0.99 (0.96, 1.01)	1.00 (0.97, 1.03)	0.99 (0.96, 1.02)	1.00 (0.96, 1.02)
PFDeA				
Tertile 2 (0.03-0.04)	1.03 (1.00, 1.05)	1.03 (1.00, 1.05)	1.02 (0.99, 1.05)	1.03 (1.00, 1.07)
Tertile 3 (0.05)	1.00 (0.98, 1.03)	1.01 (0.99, 1.04)	0.99 (0.95, 1.02)	1.02 (0.98, 1.06)
PFNA				
Tertile 2 (0.10-1.40)	1.02 (0.99, 1.04)	1.02 (0.99, 1.04)	1.03 (0.99, 1.06)	1.02 (0.98, 1.05)
Tertile 3 (1.50)	1.00 (0.98, 1.03)	1.01 (0.99, 1.04)	1.03 (0.99, 1.07)	1.02 (0.98, 1.06)
PFOS				
Tertile 2 (9.50-15.10)	1.01 (0.98, 1.03)	1.01 (0.98, 1.03)	1.01 (0.98, 1.05)	1.02 (0.99, 1.04)
Tertile 3 (15.20)	1.00 (0.98, 1.03)	1.01 (0.98, 1.03)	1.02 (0.99, 1.06)	1.02 (0.99, 1.06)
PFOA				
Tertile 2 (2.50-4.10)	0.97 (0.95, 1.00)	0.98 (0.95, 1.01)	0.95 (0.93, 0.97)	0.95 (0.93, 0.99)
Tertile 3 (4.20)	0.98 (0.95, 1.00)	0.98 (0.96, 1.00)	0.96 (0.93, 0.98)	0.95 (0.92, 0.98)

AF indicates acceleration factor; CrI credible interval; Et-PFOSA-AcOH, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; LOD, limit of detection; Me-PFOSA-AcOH, 2-(N-methyl-perfluorooctane sulfonamido) acetate; PFAS, perfluoroalkyl substance; PFDeA, perfluorodecanoate; PFNA, perfluorononanoate; PFOSA, perfluorooctane sulfonamide; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; Q3, 75th percentile.

PFOSA and Et-PFOSA-AcOH concentrations were dichotomized *a priori* as above/below the LOD and separately above/below Q3, respectively. All other PFASs were categorized *a priori* into tertiles.

AF represents the relative change in menstrual cycle length compared to the reference group (lowest PFAS concentration, not shown), conditional on the random effect. An AF>1 represents an increase in cycle length while an AF<1 represents a decrease in cycle length.

^aSeparate models were run for each PFAS.

^bOne model was run incorporating all PFASs.

^cAssociations adjusted for female age, BMI, and active smoking at enrollment.

^dAssociations adjusted for remaining PFASs.

^eAssociations adjusted for female age, BMI, active smoking at enrollment and remaining PFASs.

Table 4

Associations Between Serum PFAS and Day-Specific Probability of Pregnancy With Adjustment for Cycle Length Estimated Using Bayesian Joint Model, LIFE Study, 2005-2009.

Covariate	Single PFAS Model ^a		Multiple PFAS Model ^b	
	Adjusted ^c OR (95% CrI)	Adjusted ^d OR (95% CrI)	Adjusted ^e OR (95% CrI)	Adjusted ^f OR (95% CrI)
Cycle Length				
Linear (days)	1.5 (1.1, 2.1)	1.4 (1.0, 1.9)	1.5 (1.1, 2.1)	1.5 (1.1, 2.2)
Quadratic (days ²)	0.7 (0.5, 0.8)	0.7 (0.6, 0.9)	0.7 (0.6, 0.9)	0.8 (0.6, 0.9)
PFAS (ng/mL)				
PFOSA				
LOD (0.10)	0.6 (0.4, 1.1)	0.6 (0.3, 1.1)	0.6 (0.3, 1.1)	0.6 (0.3, 1.0)
Et-PFOSA-AcOH				
Q3 (0.06)	0.8 (0.5, 1.2)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)
Me-PFOSA-AcOH				
Tertile 2 (0.02-0.03)	1.1 (0.7, 1.6)	1.1 (0.7, 1.7)	1.1 (0.7, 1.6)	1.1 (0.7, 1.6)
Tertile 3 (0.04)	0.9 (0.6, 1.4)	1.0 (0.7, 1.4)	1.0 (0.6, 1.5)	1.0 (0.6, 1.5)
PFDeA				
Tertile 2 (0.03-0.04)	0.7 (0.5, 1.1)	0.7 (0.5, 1.1)	0.9 (0.5, 1.5)	0.9 (0.5, 1.4)
Tertile 3 (0.05)	0.9 (0.6, 1.2)	0.9 (0.6, 1.3)	1.4 (0.7, 2.6)	1.4 (0.7, 2.5)
PFNA				
Tertile 2 (0.10-1.40)	0.7 (0.4, 1.0)	0.7 (0.5, 1.0)	0.6 (0.4, 1.1)	0.6 (0.4, 1.0)
Tertile 3 (1.50)	0.8 (0.5, 1.1)	0.8 (0.6, 1.2)	0.7 (0.4, 1.3)	0.7 (0.3, 1.1)
PFOS				
Tertile 2 (9.50-15.10)	1.0 (0.6, 1.4)	1.0 (0.7, 1.5)	1.3 (0.8, 2.0)	1.2 (0.8, 1.8)
Tertile 3 (15.20)	0.9 (0.6, 1.2)	0.9 (0.6, 1.3)	1.1 (0.7, 1.9)	1.1 (0.7, 1.8)
PFOA				
Tertile 2 (2.50-4.10)	0.9 (0.6, 1.4)	1.0 (0.7, 1.5)	1.0 (0.6, 1.6)	1.1 (0.7, 1.7)
Tertile 3 (4.20)	0.7 (0.5, 1.0)	0.7 (0.5, 1.1)	0.7 (0.4, 1.2)	0.7 (0.4, 1.2)

CrI indicates credible interval; Et-PFOSA-AcOH, 2-(N-ethyl-perfluorooctane sulfonamido) acetate; LOD, limit of detection; Me-PFOSA-AcOH, 2-(N-methyl-perfluorooctane sulfonamido) acetate; OR, odds ratio; PFAS, perfluoroalkyl substance; PFDeA, perfluorodecanoate; PFNA, perfluorononanoate; PFOSA, perfluorooctane sulfonamide; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoate; Q3, 75th percentile. PFOSA and Et-PFOSA-AcOH concentrations were dichotomized *a priori* as above/below the LOD and separately above/below Q3, respectively. All other PFASs were categorized *a priori* into tertiles.

OR is the exponentiated coefficient in the model for day-specific probability of pregnancy. For the PFASs, the reference group is the group with the lowest PFAS concentration (not shown).

^aSeparate models were run for each PFAS.

^bOne model was run incorporating all PFASs.

^cAssociations adjusted for couple intercourse pattern and female menstrual cycle length.

^dAssociations adjusted for couple intercourse pattern, female menstrual cycle length, age, BMI, and active smoking at enrollment.

^eAssociations adjusted for couple intercourse pattern, female menstrual cycle length and remaining PFASs.

^fAssociations adjusted for couple intercourse pattern, female menstrual cycle length, age, BMI, active smoking at enrollment and remaining PFASs.

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