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Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility

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Abstract

Perfluorinated chemicals (PFCs) can act as endocrine-disrupting chemicals, but there has been limited study of their effects on ovarian reserve or fecundability. 99 women, 30-44 years old, without infertility were followed until pregnancy. Initially, serum was evaluated for Antimüllerian hormone (AMH), thyroid hormones: thyroid stimulating hormone (TSH), thyroxine (T4), free thyroxine (fT4), and triiodothyronine (T3), and PFCs: perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS). Bivariate analyses assessed the relationship between thyroid hormones, AMH, and PFCs. Fecundability ratios (FR) were determined for each PFC using a discrete time-varying Cox model and a day-specific probability model. PFC levels were positively correlated with each other (r 0.24 to 0.90), but there was no correlation with TSH (r 0.02 to 0.15) or AMH (r -0.01 to -0.15). FR point estimates for each PFC were neither strong nor statistically significant. Although increased exposure to PFCs correlates with thyroid hormone levels, there is no significant association with fecundability or ovarian reserve.

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Keywords

fecundability; perfluorinated chemicals; endocrine disrupting chemicals; ovarian reserve; thyroid hormones

Introduction

Endocrine disruptors are chemicals that impact the normal endocrine system leading to impaired developmental, immune, and reproductive function (1). There are many types of endocrine disrupting chemicals (EDCs), including chemicals found in nature and others that are man-made. One class of EDCs is the perfluorinated chemicals (PFCs), which have been widely used in consumer goods including food packaging, paper wraps, firefighting foams, pesticides, textiles, industrial surfactants and emulsifiers, and Teflon (1-3). In addition, PFCs have also been found as a contaminant in food and drinking water sources (4-6). PFCs are characterized by a fully fluorinated linear carbon chain attached to a hydrophilic head (2). Due to their structure, PFCs are highly resistant to chemical and thermal degradation and are persistent in the environment, with an estimated mean half-life in humans of approximately 3.8 years for perfluorooctanoate (PFOA), 5.4 years for perfluorooctanesulfonate (PFOS), and 8.5 years for perfluorohexanesulfonate (PFHxS) (7). The ability for these compounds to persist in the environment could lead to substantial exposure and therefore, potentially affecting human health.

Studies on the impact of perfluorinated chemicals on reproductive end points have reported mixed outcomes (8, 9). Large retrospective pregnancy cohort studies have shown an association between higher levels of PFOA and PFOS and an increased time to pregnancy, but are limited due to the retrospective nature of the investigation (10-12). Prospective observations have not revealed a clear association between higher exposure to PFCs and a decline in fecundability, although in most analyses no association has been seen (13, 14). Animal studies have suggested that PFCs may alter cholesterol metabolism (needed for sex steroid synthesis) and thyroid hormone levels, leading to abnormal hormonal profiles in those exposed (3, 15-17); however, further causative factors have not been explored. It is possible that PFCs could negatively impact ovarian reserve. Although studies on the impact of Antimüllerian hormone (AMH), a measure of ovarian reserve, on fecundability has yielded inconsistent results (18, 19), AMH does appear to be associated with decline in fertility and age of natural menopause (20, 21). As PFC exposure has been associated with an earlier age of menopause (22, 23), it is possible that women exposed to higher levels of PFCs have also exhibit diminished ovarian reserve, as evidenced by lower AMH levels.

Thyroid dysfunction has also been shown to adversely impact fecundity. Thyroid hormone dysfunction can lead to menstrual abnormalities, infertility and pregnancy loss (24-27). Studies in animals and humans show that PFCs can alter thyroid hormones. Although human epidemiologic studies have not yielded consistent results regarding the exact relationship between PFCs and thyroid hormones, it appears that elevated PFOA and PFOS levels result in disruption of thyroid hormone balance (28-30). However, the relationship between PFCs, thyroid hormone levels, and fertility has not been explored.

The objective of this study was to determine the extent to which PFCs are associated with thyroid function, AMH as a marker of ovarian aging, and natural fertility in women over 30 years of age. We hypothesized that greater exposure to perfluorinated chemicals could be associated with thyroid dysfunction and decreased ovarian reserve, which would result in lower fecundability.

Methods

Study design and cohort

Time to Conceive, a time-to-pregnancy cohort study, was approved by the institutional review board of the University of North Carolina. English-speaking women between 30-44 years of age, who were attempting to conceive for 3 months or less, living in the Raleigh, Durham, and Chapel Hill, NC area were eligible for participation. Women with a history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded from participation. Eligible women were enrolled and provided informed consent at their initial study visit, scheduled on the second, third, or fourth menstrual day (first day defined as the first day of bleeding). Women who were using contraception were enrolled in the menstrual cycle immediately following cessation of birth control.

Data and biospecimen collection

Serum samples were collected between November 2008 and September 2009. At the initial study visit, participants were provided with and instructed on the use of the study diary, which was designed to collect information on vaginal bleeding, intercourse, pregnancy test results, and medication use. Participants were asked to complete the diary daily until pregnancy detection or completion of three menstrual cycles. In addition, women were provided with free home pregnancy tests, with a sensitivity of 20 mIU human chorionic gonadotropin (hCG) per mL, and were directed on appropriate time for usage of these tests. Women were informed to notify study staff of a positive pregnancy test and provided a free pregnancy ultrasound to encourage notification. Women who did not report a positive test were contacted at 3 months and 6 months after the initial study visit. Women were followed until a positive pregnancy test or until 6 months of attempting to conceive after the initial study visit.

At the study visit, women also provided a blood sample. Serum samples were frozen and stored at -80 degrees Celsius until analysis. Serum was shipped 1) to the University of Southern California Reproductive Endocrinology Laboratory where the samples were assayed for Antimüllerian hormone (AMH), 2) to the National Institute of Environmental Health Sciences, National Toxicology Program Laboratory, where the samples were assayed for thyroid hormones: thyroid stimulating hormone (TSH), thyroxine (T4), free thyroxine (fT4), and triiodothyronine (T3), and 3) to the U.S. Environmental Protection Agency, National Exposure Research Laboratory, where samples were assayed for the PFCs: PFOA, PFOS, perfluorononanoic acid (PFNA), and PFHxS.

Chemical analysis

AMH concentration was measured using monoclonal two site ELISA (Gen II AMH Assay, Beckman-Coulter). AMH concentrations are reported as nanograms per milliliter (ng/mL). Serum thyroid hormone analyses were measured in duplicate by radioimmunoassay (RIA); purchased from Siemens Healthcare Diagnostics (Los Angeles, CA). Samples were analyzed on an Apex Automatic Gamma Counter (ICN Micromedex Systems, Inc., Huntsville, AL). Thyroid hormone concentrations are reported as micro-International units per milliliter ($\mu\text{IU/mL}$) for TSH, nanograms per deciliter (ng/dL) for T3 and fT4, and micrograms per deciliter ($\mu\text{g/dL}$) for T4. The limits of detection for the assays were $0.17 \mu\text{IU/mL}$ TSH, 20 ng/dL T3, $1 \mu\text{g/dL}$ T4, and 0.1 ng/dL fT4. Serum PFC concentrations were measured using liquid chromatography tandem mass spectrometry for quantification, following standard procedures previously outlined and described (31). All PFCs are reported in nanograms per milliliter. The limits of detection for the assays were 0.25 ng/mL PFOA, 1 ng/mL PFOS, 0.50 ng/mL PFNA, and 0.5 ng/mL PFHxS. Interassay coefficients of variation ranged from 7% to 11%. When samples were below the level of detection, they were imputed at the LOD divided by the square root of 2, as per standard practice (32).

Statistical analysis

In descriptive analysis, geometric means (GMs) and 95% confidence intervals (CIs) were calculated for each PFC and stratified by 1) parity at study start and 2) pregnancy at the end of the study. Differences between mean PFC levels based on pregnancy and parity were assessed using the nonparametric Wilcoxon test. Bivariable analyses were conducted to evaluate the relationship between covariates and sum PFC exposure (defined as women in the upper quartile of sum PFC levels). Student's t-test and the chi-square test were used for continuous and categorical variables, respectively. All analyses were carried out with the use of STATA statistical software (version 13.0; StataCorp LP, College Station, TX).

Pearson's correlation coefficients and P values were used to evaluate the relationship between AMH, thyroid hormones, and PFCs, excluding four women taking medication for thyroid disease. Variables which were not normally distributed were normalized before measuring correlation. Specifically, AMH (ng/mL), TSH ($\mu\text{IU/mL}$) and each PFC (ng/mL) were log transformed. A linear regression model was then used to further evaluate the associations between PFC exposure levels and ovarian reserve and thyroid hormones, both unadjusted and adjusted for age. This model also used log transformed results for AMH (ng/mL), TSH ($\mu\text{IU/mL}$), and each PFC (ng/mL) as they were not normally distributed.

Subsequently, we evaluated the association between the PFCs of interest and fecundability. The probability of pregnancy per cycle (fecundability) ratio was determined using both a cycle-specific and a day-specific probability of pregnancy model. A fecundability ratio (FR) less than 1.0 suggests reduced fecundability. Pregnancy was defined by the report of a positive home pregnancy test. As there are no standard level of PFC levels used in clinical practice, cut-off points were based on quartiles of the data with the upper quartile considered exposed (33). Specifically, we dichotomized each PFC at the upper quartile, as follows: PFOA at 3.68 ng/mL, PFOS at 13.52 ng/mL, PFNA at 1.22 ng/mL, and PFHxS at 2.73 ng/mL. In order to evaluate the association between cumulative PFC exposure, sum PFC

levels were evaluated (sum of PFOA, PFOS, PFNA, and PFHxS levels), with dichotomization at the upper quartile of exposure (20.64 ng/mL). Due to the relatively small sample size, the only covariates used in adjusted analysis included maternal age, dichotomized at 35 years of age, and mean cycle length (in days).

Model 1 (cycle-specific) utilized a discrete-time Cox proportional hazards model to determine the impact of exposure to each PFC on fecundability. This model accounts for both right censoring and left truncation (due to women enrolling in cycles 1, 2, 3 or 4 of their pregnancy attempt), which were present in the data. Parity was evaluated in this model as a potential effect modifier, with no significant interaction seen with PFC exposure, and thus parity was not included in the model. In addition to the unadjusted model, adjusted models were created with 1) age alone (“adjusted model”) and 2) age and mean cycle length (“fully adjusted model”) for each PFC of interest.

Model 2 (day-specific) utilized information from the daily diary on days of menstrual bleeding, days of intercourse, and results of pregnancy tests to estimate day-specific probabilities of pregnancy (probability of pregnancy given an act of intercourse on a fertile day). Ovulation was assumed to have occurred 14 days before either the first day of menses or positive pregnancy test, with the fertile window defined as 6 days before until 5 days after ovulation (34). The day specific probabilities model by Scarpa and Dunson was used to generate day-specific FRs (35). This model accommodates multiple days for which intercourse occurred and assumes independence between acts of intercourse. For this model, the first observed menstrual cycle is entered at the appropriate attempt time (cycle 1 for those who enrolled when they initially started trying to conceive and cycles 2-4 for those who enrolled later in their attempts). Prospectively observed cycles were included in the model for up to three observed cycles or until pregnancy detection. We fit both unadjusted models and age-adjusted models for each PFC of interest.

Results

The analysis included 99 women, with a total of 328 cycles evaluated with model 1 and 185 cycles evaluated with model 2. Blood samples for 99 women who were enrolled in the study were analyzed, and complete follow-up was available for 98 women. Thirty-one percent of women were 35 years or older, and 4% were between 40-44 years of age. Participants tended to be parous (60%), Caucasian (87%), highly educated (64% with at least some graduate degree work), and with a normal body mass index (62% between 18.5 and 24.9; calculated as weight (kg)/[height (m)]²). Sixty-seven percent of women in the study became pregnant, with 12% of women conceiving in the first observed cycle (Table 1). Women in the highest quartile of sum PFC exposure were overall similar to women with lower exposure levels. However, women in the highest exposure group were more likely to have longer mean cycle lengths (30.7 versus 28.7 days, $p=0.02$) and less likely to achieve pregnancy at study end (54% versus 75%, $p=0.04$) as compared to women with lower sum PFC exposures.

Table 2 presents the GMs and 95% CIs for the PFC measurements in our cohort. The GMs (ng/mL) for PFOA, PFOS, PFNA, and PFHxS were 2.79 (95% CI: 2.48-3.16), 9.29 (95% CI: 8.31-10.38), 0.84 (95% CI: 0.74-0.97), and 1.59 (95% CI: 1.37-1.84), respectively. GMs

were also stratified by parity at study start, and there was a trend for decreased mean serum levels of all PFCs in parous compared to nulliparous women. Significantly decreased serum concentrations of PFOA and PFHxS were noted in parous women compared to nulliparous women ($p<0.01$ and $p=0.04$, respectively). Pregnancy status at study end had no bearing on the GMs of any PFC.

Correlations between thyroid hormones, AMH, and each PFC are presented in Table 3. AMH was not correlated with any PFC. Thyroid hormones were weakly correlated with PFCs. Specifically, PFOA and PFNA were positively correlated with T3 ($r=0.23$, $p=0.03$), PFNA was positively correlated with free T4 ($r=0.24$, $p=0.02$), but there was no correlation with TSH. The PFCs were positively correlated with each other (r values ranging 0.24 to 0.90, p -values <0.02). Table 4 presents associations between each PFC with AMH and thyroid hormones. Beta coefficients are presented corresponding to the change in each respective hormone with each unit (ng/mL) change in lnPFC (for each respective PFC), both unadjusted and adjusted for age. Similar to the findings using correlations, some thyroid hormones (T3 and free T4) were associated with PFOA, PFNA, and sum PFC levels, but no association was seen between PFC levels and AMH. An age-adjusted regression model yielded similar results.

Both unadjusted and adjusted cycle-specific and day-specific fecundability ratios were neither strong nor significantly associated with PFC exposure (Table 5). In cycle-specific analysis, fully-adjusted FRs (95% CI) for PFOA, PFOS, PFNA, and PFHxS were 1.15 (0.66-2.01), 0.89 (0.49-1.60), 0.84 (0.46-1.54), and 1.40 (0.79-2.49), respectively. In day-specific analysis, adjusted FRs (95% CI) for PFOA, PFOS, PFNA, and PFHxS were 0.96 (0.31-1.94), 0.99 (0.28-2.32), 0.98 (0.32-2.10) and 0.96 (0.31-1.71), respectively. In evaluation of sum PFC levels, there was no significant association with fecundability in either model, with an adjusted FR of 0.67 (0.36-1.26) and 1.05 (0.35-2.51) in the cycle-specific model and day-specific models, respectively.

Discussion

PFC levels in our cohort were consistent with levels seen in the general US population of reproductive age women (36). There was positive correlation between PFCs measured in this study. Although PFC levels were correlated with thyroid hormone levels, there was not a strong nor a significant association with ovarian reserve (AMH as a proxy). In our cohort, fecundability was not associated with serum PFC levels.

Recent National Health and Nutrition Examination Survey (NHANES) data reveals that PFC levels have declined overall since 1999 (36). The levels of exposure seen in our cohort were similar to what is reported nationwide for females of reproductive age in 2007-2008. Specifically, U.S.-wide GMs (ng/mL) and 95% CI were calculated for PFOA, PFOS, PFNA, and PFHxS at 3.56 (3.38-3.74), 10.7 (9.72-11.8), 1.33 (1.20-1.47), and 1.46 (1.30-1.64), respectively (36). PFC exposure can vary based on geographic region, and von Ehrenstein evaluated PFC levels in breastfeeding women in North Carolina from 2004-2005, with mean PFC levels (ng/mL) at initial study visit of 3.86 PFOA, 21.9 PFOS, 1.19 PFNA, and 1.94 PFHxS (37). Our cohort, similarly with women of reproductive age, had lower PFC

exposure levels from samples taken 4-5 years after the von Ehrenstein study. In addition, in comparison to prior studies evaluating fertility and PFC exposure, mean levels of PFOA and PFOS were similar to that in the only other U.S. investigation including PFCs and fecundability (13). Our cohort also had similar mean PFC levels to that reported in Canadian and Norwegian pregnancy cohorts, but much lower levels of PFOA and PFOS than that seen in Danish studies (10-12, 14). Despite the overall decrease in PFC serum levels since 1999, our data support previously reported findings that PFCs are still prevalent in our environment at measurable concentrations.

Exposure to one PFC was positively correlated with exposure to other PFCs. This suggests that women in our cohort likely have joint exposure pathways to PFCs as a class. Similarly, in an evaluation of Danish children and their mothers (n=143), strong and significant positive correlations between PFCs were found in both mothers and children (38). In addition, NHANES data (n=1,181) revealed similar positive correlations between PFC levels as a class in the U.S. population (29). The Norwegian Mother and Child Cohort Study (n=891) also revealed strong and significant positive associations between PFCs in pregnant women (39). Our results support these prior findings of a joint exposure pattern, where increased exposure to one PFC is related to increased exposure to other PFCs.

Although prior epidemiologic studies on PFCs and thyroid function have reported mixed results, our analysis indicates correlations between PFCs and thyroid hormone levels. Specifically, we found the same positive correlations between PFOA and T3 as was seen in a recent NHANES evaluation by Wen et al. (n=1,181) from 2007-2010 (29). We also found positive correlations between PFNA with T3 and free T4 levels, which is similar to findings reported in a Taiwanese cohort of young adults (30). In addition, we found no correlation between TSH levels and any of the 4 PFCs evaluated, or sum PFC levels, also consistent with prior reports (29, 30). We could not draw conclusions based on our data regarding the potential association between PFCs and thyroid disease. However, prior NHANES evaluations of the US population previously revealed associations between higher levels of PFC exposure and an increased incidence of both subclinical and overt thyroid disease (28, 29, 40). Thus, thyroid hormone modulation may be a mechanism by which PFC levels could impair optimal reproductive function.

Our study is the first to evaluate the potential association between PFC exposure and ovarian reserve. Although no association was seen between PFC exposure and AMH levels, prior epidemiologic studies have revealed an association between PFC exposure and earlier age of menopause (22, 23). A recent publication using NHANES data from 1999-2010 (n=2,732) revealed an association between the highest concentrations of PFCs (PFOA, PFOS, PFNA, and PFHxS) and an earlier age of natural menopause (22). In addition, a cross sectional analysis of women from the C8 Health Project (n=25,957), which uses data from six public water districts known to be contaminated with PFOA between 2005-2006, also revealed associations between higher PFC exposure (PFOA, PFOS) and earlier menopause (23). Of note, as these are cross sectional evaluations, only associations can be seen and not conclusions about causality. However, diminished ovarian reserve, as evidenced by lower AMH levels, has been linked to an earlier age of natural menopause (20, 21, 41, 42). Thus, if higher PFC exposure does result in an earlier age of menopause, it is possible that ovarian

reserve may be impacted as well. Further, this evaluation of women, with a mean age of 33 years, may not be ideal to assess the impact of PFC exposure on ovarian reserve.

In our prospective cohort, there was no significant association between higher PFC exposure levels and fecundability. Previous studies attempting to evaluate fecundability and PFC exposure have demonstrated no consistent result. In retrospective analysis, increased levels of PFCs have been associated with recall of increased time to pregnancy. In a study utilizing the Danish National Birth Cohort, Fei et al. asked pregnant women (n=1240) to recall time to pregnancy, and higher levels of PFOA and PFOS were significantly associated with increased time to pregnancy (10). However, in a recent reanalysis of this data, no association was seen with PFC levels and fecundability (43). The Norwegian Mother and Child Cohort Study (n=891) did reveal an association between highest level of PFOA and PFOS exposure with subfecundity in parous women, although this association was not seen in nulliparous women (11). In addition, in a Canadian cohort pregnant women (n=1743), Velez et al. asked women via questionnaire to self-report the number of months of unprotected intercourse prior to becoming pregnant and found an association between increased levels of PFOA and PFHxS in pregnancy and an increased time to conception (12). However, inherent in evaluation of fecundability from a pregnancy cohort is the potential bias to not see the strongest possible correlation if the exposure resulted in infertility. In the prospective analyses evaluating fecundability and PFC exposure, no association has been clearly established. Vestergaard et al. prospectively evaluated Danish couples (n=222), attempting pregnancy for the first time, with no significant association seen between PFC levels (PFOA, PFOS, PFNA, and PFHxS) and fecundability (14). Further, in a prospective U.S. cohort of couples attempting pregnancy (n=501), Buck Louis et al. found no significant association between PFOA and PFOS and cycle-specific fecundability although an association was seen with perfluorooctane sulfonamide, (PFOSA) (13). The discordant findings may be partly explained by the association between parity and PFC levels. Unlike prospective evaluations, parity has the potential for reverse-causality in evaluating subfecundability or an increased time to pregnancy in retrospective studies (8). Our findings support those of previous prospective studies reporting no clear association between higher levels of PFCs and a fecundability.

Consistent with prior reports, our study population at baseline did exhibit reduced levels of PFCs in parous women as compared to nulliparous. Most data attribute this finding to a transfer of PFC body burden during pregnancy, as evidenced by umbilical cord blood samples at birth demonstrating detectable PFC levels (44-48). Further, breastfeeding has also been suggested as a potential transfer mechanism of PFCs from mother to infant, with a decrease in maternal concentrations seen with an increase in duration of breastfeeding, as well as a positive association between serum PFOA and length of time breastfed in children (46, 49, 50). Supporting these findings, in an evaluation from a Norwegian birth cohort (n=391), nulliparous women had significantly higher levels of PFCs than did parous women (51). Although there was a difference in PFC levels when stratified by parity at study entry in our cohort, determining how to incorporate parity into an evaluation of fecundability is difficult. Thus, many different strategies have been used in previous studies when comparing fecundability and PFC exposure, including adjusting for parity, stratification by parity, and separate sensitivity analyses. As parity and fecundability are intrinsically linked, adjusting or

stratifying for parity in a model may mask potential findings by over adjustment, and thus we have decided not to include parity in our model (52). We did, however, evaluate parity in an exploratory analysis as an effect modifier, and found no significant differences in fecundability. Certainly parity has the potential for reverse causality in retrospective studies, which adds to the complexity of evaluating the relationship between PFC exposure levels and fecundability.

To our knowledge, our study is only the second prospective evaluation of PFC exposure and fecundability in a U.S. population and is the first study to look at the potential association between PFC levels and ovarian reserve. Our study does have limitations. The cohort was mostly Caucasian, well-educated, and older women. Our findings may not be generalizable to other groups. With a mean age of 33 years, our study population is older than the general population trying to conceive, but still relatively young. This may make our ability to see associations with ovarian reserve more difficult. Further, this was an exploratory study in a sub-set of patients from the Time to Conceive cohort, and our sample size was limited. Thus, it is possible that we may be lacking the power to detect a small change in fecundability. Our follow-up time period was only 6 months, thus we may also be limiting our ability to fully detect an association with fecundability. In addition, there is no standard practice on how to statistically evaluate PFC concentrations. Previous studies have included dividing the exposure into quartiles, above and below the median, and as a continuous log-transformed variable (10, 12-14). Our decision to compare women above the upper quartile of exposure to those below was based on our desire to see if there was an impact at the highest levels of exposure. It is possible that a different comparison (such as the upper quartile to the lowest) may have yielded different results. Strengths of this study include the prospective nature of the study in a non-infertile population trying to conceive, which also limits the potential impact of parity to have reverse-causality. In comparison to studies of fecundability in pregnancy cohorts, our study eliminates recall bias and is a more accurate evaluation of fecundability. In addition, using both a cycle-specific and day-specific model for evaluation of fecundability allows more confident interpretation of our findings.

Conclusions

In summary, our study reveals generally null and inconsistent associations between higher levels of individual PFCs and fecundability in a prospective cohort attempting pregnancy. Although exposure to some PFCs were correlated with thyroid hormone levels, there does not appear to be any correlation between higher levels of individual PFC exposures and ovarian reserve, as measured by AMH. As PFCs are still prevalent in our population, further research to explore the potential association between PFC exposure and fecundability should be explored as well as the mechanistic pathways involved, including the effect on reproductive hormones. In addition, the potential impact PFC exposure may have in an infertile population has yet to be investigated.

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Table 1
Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility: Characteristics of the study population

	Overall mean (SD) or % (n=99)	PFC unexposed ^I mean (SD) or % (n=71)	PFCs exposed ^I mean (SD) or % (n=28)	p-value
Age (years)	33.3 (3)	33.4 (3)	33.2 (3)	0.78
Race				0.27
Caucasian	87%	85%	93%	
Other	13%	15%	7%	
Married	97%	97%	96%	0.84
Education				
Less than college degree	7%	6%	11%	0.34
College degree	19%	15%	28%	
At least some graduate work	74%	79%	61%	
BMI (mg/kg ²)	25.2 (6)	25.1 (6)	25.3 (6)	0.92
Thyroid disease	4%	4%	4%	0.89
Parous	60%	64%	50%	0.19
Prior pregnancy loss	33%	39%	14%	0.21
Regular menstrual cycles	89%	89%	89%	0.94
Cycle length (days)	29.2 (4)	28.7 (3)	30.7 (5)	0.02 *
Partner's age	34.6 (5)	34.4 (5)	34.9 (5)	0.66
Partner's BMI (mg/kg ²)	26.8 (4)	26.8 (4)	26.8 (4)	0.97
Pregnant at study end	67%	75%	54%	0.04 *

^I PFC exposure for the purpose of bivariable analysis was defined as the upper quartile of exposure to the sum PFC variable

* p < 0.05

Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility: Geometric means (95% confidence intervals) of serum perfluorochemical concentrations (ng/ml), stratified by parity at study start and pregnancy at study end

Table 2

	LOD	n < LOD	Overall n=99	Parity		Pregnancy Status	
				Nulliparous n=63	Parous n=36	Pregnant n=68	Not Pregnant n=30
PFOA	0.25	0	2.79 (2.48,3.16)	3.26* (2.79,3.80)	2.85* (2.46,3.29)	2.85 (2.46,3.29)	2.78 (2.18,3.56)
PFOS	1.00	1	9.29 (8.31,10.38)	9.46 (8.19,10.94)	8.98 (7.50,10.76)	9.21 (8.19,10.35)	9.58 (7.25,12.66)
PFNA	0.50	23	0.84 (0.74,0.97)	0.90 (0.75,1.08)	0.76 (0.62,0.93)	0.82 (0.69,0.96)	0.93 (0.73,1.19)
PFHxS	0.50	4	1.59 (1.37,1.84)	1.76* (1.46,2.12)	1.33* (1.06,1.66)	1.65 (1.39,1.93)	1.43 (1.08,1.91)

LOD, limit of detection (ng/mL); GM, geometric mean; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoic acid; PFHxS, perfluorohexanesulfonic acid.

* p < 0.05, in comparing PFC levels between nulliparous versus parous women

Table 3
Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility: Correlation between ovarian reserve, thyroid hormones, and PFCs¹

	AMH	TSH	T3	T4	Free T4	PFOA	PFOS	PFNA	PFHxS	sum PFCs
AMH	1.00									
TSH	-0.11	1.00								
T3	-0.17	0.06	1.00							
T4	-0.16	-0.02	0.60 *	1.00						
Free T4	0.01	-0.12	0.42 *	0.53 *	1.00					
PFOA	-0.04	0.15	0.23 *	0.15	0.13	1.00				
PFOS	-0.01	0.07	0.10	0.04	0.02	0.37 *	1.00			
PFNA	-0.15	0.11	0.24	0.01	0.21 *	0.55 *	0.31 *	1.00		
PFHxS	-0.08	0.02	0.12	-0.12	-0.04	0.45 *	0.36 *	0.24 *	1.00	1.00
sum PFCs	-0.06	0.10	0.20	0.06	0.09	0.69 *	0.90 *	0.53 *	0.58 *	

¹ Pearson's correlation coefficients presented

* p < 0.05

Table 4
Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility: Unadjusted and adjusted associations between PFC levels, AMH, and thyroid hormones¹

	Unadjusted associations β coefficient (p-value)					Adjusted associations ² β coefficient (p-value)				
	AMH	TSH	T3	T4	Free T4	AMH	TSH	T3	T4	Free T4
PFOA	-0.57 (0.74)	0.09 (0.37)	6.04 (0.03)*	0.47 (0.07)	0.05 (0.10)	-0.56 (0.75)	0.09 (0.37)	6.05 (0.03)*	0.47 (0.07)	0.05 (0.11)
PFOS	0.01 (0.98)	0.01 (0.93)	3.10 (0.30)	0.24 (0.40)	0.03 (0.45)	0.07 (0.73)	-0.01 (0.98)	3.94 (0.19)	0.31 (0.28)	0.03 (0.42)
PFNA	-0.18 (0.25)	0.01 (0.89)	5.52 (0.02)*	0.21 (0.37)	0.08 (<0.01)*	-0.17 (0.27)	0.01 (0.91)	5.65 (0.02)*	0.22 (0.34)	0.08 (<0.01)*
PFHxS	-0.11 (0.47)	-0.03 (0.69)	2.96 (0.20)	-0.13 (0.55)	0.01 (0.83)	-0.12 (0.41)	-0.03 (0.71)	2.80 (0.22)	-0.15 (0.50)	0.01 (0.84)
sum PFCs	-0.11 (0.63)	0.02 (0.87)	7.11 (0.04)*	0.40 (0.23)	0.06 (0.14)	-0.06 (0.78)	0.01 (0.94)	7.80 (0.03)*	0.46 (0.17)	0.07 (0.13)

PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoic acid; PFHxS, perfluorohexanesulfonic acid; PFC, perfluorinated chemicals;

¹ β coefficients and p-values presented from a regression analysis

² adjusted for age

* p<0.05

Effects of perfluorinated chemicals on thyroid function, markers of ovarian reserve, and natural fertility: Fecundability Ratio (FR) (95% Confidence Intervals) for each PFC

	Cycle-specific model ¹		Day-specific model ²		
	Unadjusted FR	Adjusted FR ³	Fully adjusted FR ⁴	Unadjusted FR	Adjusted FR ³
PFOA	1.20 (0.69,2.10)	1.16 (0.69,2.02)	1.15 (0.66,2.01)	0.95 (0.25,2.08)	0.96 (0.31,1.94)
PFOS	0.91 (0.51,1.63)	0.87 (0.49,1.56)	0.89 (0.49,1.60)	1.03 (0.30,2.45)	0.99 (0.28,2.32)
PFNA	0.78 (0.43,1.42)	0.80 (0.43,1.45)	0.84 (0.46,1.54)	0.91 (0.23,1.66)	0.98 (0.32,2.10)
PFHxS	1.46 (0.84,2.55)	1.42 (0.81,2.47)	1.40 (0.79,2.49)	0.95 (0.31,1.71)	0.96 (0.31,1.71)
sum PFC	0.78 (0.44,1.41)	0.74 (0.41,1.33)	0.67 (0.36,1.26)	1.01 (0.32,2.17)	1.05 (0.35,2.51)

upper quartile of each PFC considered exposed

PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoic acid; PFHxS, perfluorohexanesulfonic acid; PFC, perfluorinated chemicals

¹Model 1: based on a Cox proportional hazards model

²Model 2: based on a day-specific probability model

³adjusted for age

⁴adjusted for age and mean cycle length