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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 Antimullerian 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 0.24–0.90), but there was no correlation with TSH (r 0.02–0.15) 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.

1. 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

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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.

2. Methods

2.1. 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 and 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.

2.2. 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° Celsius until analysis. Serum was shipped (1) to the University of Southern California Reproductive Endocrinology Laboratory where the samples were assayed for Antimullerian 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.

2.3. 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 Micromedic Systems, Inc., Huntsville, AL). Thyroid hormone concentrations are reported as micro-International units per milliliter (μIU/mL) for TSH, nanograms per deciliter (ng/dL) for T3 and fT4, and micrograms per deciliter (μg/dL) for T4. The limits of detection for the assays were 0.17 μIU/mL TSH, 20 ng/dL T3, 1 μg/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].

2.4. 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 (version13.0; 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 (μIU/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 (μIU/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 expo-