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Perfluorinated compounds and subfecundity in pregnant women

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Abstract

Background—Perfluorinated compounds are ubiquitous pollutants; epidemiologic data suggest they may be associated with adverse health outcomes, including subfecundity. We examined subfecundity in relation to two perfluorinated compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Methods—This case-control analysis included 910 women enrolled in the Norwegian Mother and Child Cohort Study in 2003 and 2004. Around gestational week 17, women reported their time to pregnancy and provided blood samples. Cases consisted of 416 women with a time to pregnancy greater than 12 months, considered subfecund. Plasma concentrations of perfluorinated compounds were analyzed using liquid chromatography-mass spectrometry. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each pollutant quartile using logistic regression. Estimates were further stratified by parity.

Results—The median plasma concentration of PFOS was 13.0 ng/ml (interquartile range [IQR]=10.3-16.6 ng/ml) and of PFOA was 2.2 ng/ml (IQR=1.7-3.0 ng/ml). The relative odds of subfecundity among parous women was 2.1 (95% CI=1.2-3.8) for the highest PFOS quartile and 2.1 (1.0-4.0) for the highest PFOA quartile. Among nulliparous women, the respective relative odds were 0.7 (0.4-1.3) and 0.5 (0.2-1.2).

Conclusion—Previous studies suggest that the body burden of perfluorinated compounds decreases during pregnancy and lactation through transfer to the fetus and to breast milk. Afterwards, the body burden may rise again. Among parous women, increased body burden may be due to a long interpregnancy interval rather than the cause of a long time to pregnancy. Therefore, data from nulliparous women may be more informative regarding toxic effects of perfluorinated compounds. Our results among nulliparous women did not support an association with subfecundity.

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Perfluorinated and polyfluorinated compounds (PFCs) are man-made chemicals produced since the 1950s and extensively used in a wide range of industrial and consumer applications, including polymers, repellents, surfactants, adhesives, food packaging, and fire-fighting foams.¹ These compounds are characterized by a hydrophilic head moiety attached to a hydrophobic carbon chain of varying length that is saturated with fluorine atoms (perfluorinated).² Due to their extreme resistance to degradation and potential to bioaccumulate, PFCs have been found in practically all environmental media and biota worldwide, including humans.^{3,4} PFCs have been found in non-occupationally exposed adults, children, cord blood, and human breastmilk.⁵⁻⁹ The most widely studied PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Their half-lives in humans have been determined in retired workers from PFC production facilities, with a median of 4.6 years for PFOS and 3.4 years for PFOA.¹⁰ Due to the environmental behavior and toxicity of PFCs, the major producer of PFOS has phased out its production, and its use has been restricted under the Stockholm Convention on Persistent Organic Pollutants (<http://chm.pops.int>). Further measures have been introduced to reduce industrial emissions of PFOA.¹¹ Following these measures, studies have demonstrated a decrease in body burdens of PFOS and PFOA since around the year 2000.^{12,13} Nevertheless, human health concerns regarding exposure to low levels of PFCs persist.

The toxicity of PFOS and PFOA has been extensively studied in experimental animals, with hepatotoxicity, developmental toxicity, immunotoxicity, hormonal effects, and carcinogenicity identified as the effects of most concern.^{14,15} In contrast, epidemiologic studies of the relation between PFCs and various health outcomes are limited and inconsistent. A recent study of pregnant women in the Danish National Birth Cohort linked maternal serum concentrations of PFCs with subfecundity,¹⁶ finding increased relative odds of subfecundity among women in the highest quartile of both PFOS (odds ratio [OR]=1.8 [95% confidence interval (CI)=1.1-3.0]) and PFOA (2.5 [1.5-4.4]).

A woman's pregnancy history and previous duration of breastfeeding may be important determinants of her PFC body burden and, therefore, influence her PFC plasma concentration.^{17,18} PFOS and PFOA have been quantified in cord blood, demonstrating their ability to cross the placental barrier.^{5,8,9} Studies have also documented declining maternal levels of PFOS and PFOA during pregnancy^{5,9} and declining maternal levels of PFOA following delivery.⁵ Further, PFCs have been detected in breast milk⁷, a recent study among primarily breastfed infants reported lower maternal PFOS and PFOA concentrations six months after birth compared with pregnancy levels.⁵ Further, the PFC body burden among parous women will be affected by the elapsed time since the previous birth (interpregnancy interval). The longer the interpregnancy interval, the greater amount of time there will be for PFC levels to increase toward prepregnancy baseline following the previous birth. The interpregnancy interval is also a reflection, in part, of the woman's underlying fecundability, which is the construct being measured by time to pregnancy. It is possible among parous women for an association between PFC levels and time to pregnancy, to be due to reverse causality. That is, parous women with long times to pregnancy have higher PFC levels because they also have long interpregnancy intervals. In the previous Danish study of PFC levels and subfecundity, separate effect estimates for parous and nulliparous women were not provided. Due to the potential for reverse causality, it is important to distinguish between women with or without a prior pregnancy. The goal of the present study was to examine the relation between PFOS and PFOA and subfecundity, with separate analyses for parous and nulliparous women.

Methods

This study is based on the Norwegian Mother and Child Cohort Study conducted by the Norwegian Institute of Public Health, with enrollment from 1999-2008.¹⁹ The majority of pregnant women in Norway were invited to participate in conjunction with a routine ultrasound exam around 17 weeks of gestation. A total of 39% of invited women participated in the study. Further details can be found at www.fhi.no/morogbarn. The Norwegian Mother and Child Cohort Study was approved by The Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate, and informed consent was obtained from each participant.

At the time of enrollment, women completed a questionnaire regarding demographic and lifestyle factors, and medical and reproductive history, including breastfeeding history for previous children. In particular, women were asked whether they had planned the index pregnancy, and were asked “how many months did you have regular intercourse without contraception before you became pregnant?” Women chose “<1 month”, “1-2 months” or “3 months or more”. Women who answered “3 months or more” were further prompted to provide the number of months. Women’s responses to these questions defined their self-reported time to pregnancy. For the present study, we defined subfecundity as self-reported time to pregnancy of greater than 12 months. Additionally, information regarding the interval between women’s previous delivery and the beginning of the index pregnancy (interpregnancy interval) was obtained from the Medical Birth Registry of Norway.

Eligibility was restricted to women who delivered a live-born child and provided a plasma sample around 17 weeks of gestation. We further restricted eligibility to women enrolled from 2003 to 2004. Compared with the earliest years of the Norwegian Mother and Child Cohort Study, the volume of plasma stored for participants was greater during these years. Furthermore, levels of PFCs appear to be declining in Norway,¹² and average PFC levels were likely higher during these years compared with later years.

Among eligible women, we randomly selected 400 who planned their pregnancy and were subfecund (i.e. who reported time to pregnancy greater than 12 months). Additionally, we randomly selected 550 cohort members who reported a time to pregnancy of any duration. Nine women originally selected as cases reported a time to pregnancy longer than their interpregnancy interval in the Medical Birth Registry of Norway. Two of these women had an interpregnancy interval less than or equal to 12 months, making it clear that their time to pregnancy could not be more than 12 months; these two women were reclassified as controls and their time to pregnancy recoded as equal to their interpregnancy interval. For the other seven women, the interpregnancy interval was greater than 12 months; these women were excluded from the analysis. Eleven of the randomly-selected cohort subjects were also excluded; nine did not report planning their pregnancy and two inconsistently reported their time-to-pregnancy. The case definition was then applied to the remaining 541 subjects in the random cohort sample, resulting in 30 additional cases. The remaining 502 women in the random cohort sample plus the two women originally selected as cases met the control definition (women with a planned pregnancy and time to pregnancy no longer than 12 months) for a total of 421 cases and 511 controls. Lastly, 163 cases (39%) and 7 controls (1%) reported receiving fertility treatment for the index pregnancy; the seven control women were excluded, leaving 421 cases and 504 controls.

Maternal plasma samples were shipped from the collection site to Oslo by mail at ambient temperature. Because PFCs are chemically stable,²⁰ changes in PFC plasma concentrations while in transit are believed to be negligible. PFOS and PFOA concentrations were measured from 150 µl of plasma using high-performance liquid chromatography/tandem

mass spectrometry at the Norwegian Institute of Public Health. For quantification of PFOS, the total area of the linear and branched isomers was integrated. Further details about the analytic method have been previously published.²¹ All values of PFOS and PFOA were above the limit of quantification (0.05 ng/ml). A total of 50 quality assurance/quality control (QA/QC) samples from a single pool were analyzed in 17 sample batches alongside the case and control specimens. Each batch contained an approximately equal proportion of case and control specimens. The lab technicians were blinded to their identity, and the QA/QC samples were indistinguishable from the plasma samples from Norwegian Cohort subjects. The within- and between-batch coefficients of variation (CV) were calculated for PFOS and PFOA based on the 50 QA/QC samples. For PFOS, the within-batch CV was 4.5% and the between-batch CV was 11.3%. The within- and between-batch CVs for PFOA were 3.5% and 6.7%, respectively. PFOS and PFOA concentrations were categorized into quartiles, with the lowest quartile as the reference category.

We used logistic regression to estimate ORs and 95% CIs for each quartile of PFOS and PFOA, separately. We adjusted *a priori* for maternal age and prepregnancy body mass index (BMI). To determine the magnitude of other potential confounding, we examined the following variables using a backwards deletion strategy²²: maternal plasma albumin concentration, calendar year of blood draw, maternal smoking, maternal alcohol intake, maternal fish consumption, maternal education, paternal age, paternal education, maternal diseases (endometriosis, sexually transmitted diseases, and fallopian tube infection), menstrual cycle irregularity, and frequency of sexual intercourse. The deletion of any of these variables did not change the ORs for the association between subfecundity and PFOS (conditional on maternal age and prepregnancy BMI) by more than 10%. However, the deletion of maternal alcohol intake changed the OR for the association between subfecundity and PFOA by 10%. Therefore, all PFOS analyses are adjusted only for maternal age and prepregnancy BMI, while PFOA analyses are additionally adjusted for maternal alcohol intake. Due to missing values for covariates, the final sample size for the PFOS analyses included 416 cases and 494 controls, while the PFOA analyses included 412 cases and 488 controls. Results were also stratified by parity (nulliparous vs. parous). We chose not to assess parity as a potential confounder because parity is influenced by a woman's underlying fecundability (which will influence her time to pregnancy), and it may also affect exposure through declining PFC body burden during previous pregnancies and lactation. To compare our results with those presented in the Danish cohort,¹⁶ we performed a sensitivity analysis using the same exposure categories as in the Danish study.

Among parous women, the interval between the two most recent pregnancies (interpregnancy interval), the number of previous pregnancies, and duration of breastfeeding may have influenced measured levels of PFCs. The effect of these variables on PFC levels may either bias the association between PFCs and subfecundity (measured by self-reported time to pregnancy) or, if women with longer time to pregnancy have higher levels due only to a longer time since their previous pregnancy, result in reverse causation. To explore whether these factors have biased the association, we examined models for parous women adjusted for interpregnancy interval (after subtracting out the time to pregnancy) and breastfeeding.

Also, to further understand how the interpregnancy interval (minus time to pregnancy) and breastfeeding may have affected PFC plasma concentrations in our study, we conducted a third sensitivity analysis. Among parous women, a simple linear regression model was fit to estimate PFC plasma concentrations based on previous pregnancy variables. The dependent variable in this model was the natural log of either PFOS or PFOA. The independent variables included interpregnancy interval and duration (in months) of breastfeeding the previous child. Other factors related to the index pregnancy (maternal age at the pregnancy

attempt, prepregnancy BMI, maternal education, and maternal drinking) and parity (0, 1, or 2+ previous births) accounted for little of the variance and were not included in the final model.

The beta coefficient corresponding to the interpregnancy interval represents the change in $\ln(\text{PFC})$ levels per month, while the beta coefficient corresponding to the breastfeeding variable represents change in $\ln(\text{PFC})$ levels per month spent breastfeeding. We used these beta coefficients to explore how much of the differences in average PFC levels between cases and controls might be explained by differences in time to pregnancy and breastfeeding. By multiplying the difference in average time to pregnancy between cases and controls by the beta coefficient for the interpregnancy interval, we estimated the percent difference in average PFC levels between cases and controls due to differences in time to pregnancy. Similarly, by multiplying the difference in average months spent breastfeeding between cases and controls by the beta coefficient for breastfeeding, we estimated the percent difference in average PFC levels due to differences in breastfeeding duration. We compared these estimated values with the observed difference in PFC levels between cases and controls.

Results

At the time of the pregnancy attempt, cases tended to be younger than controls (Table 1). Also, cases had higher prepregnancy BMI, were less educated, and were more likely to report having smoked before pregnancy compared with controls. Cases were more likely than controls to have no previous pregnancies. Although the mean interpregnancy interval was the same among cases and controls, cases breastfed for a shorter amount of time than controls. Overall, the median plasma concentration of PFOS was 13.0 ng/ml (interquartile range [IQR]=10.3-16.6 ng/ml) and the median plasma concentration of PFOA was 2.2 ng/ml (1.7-3.0 ng/ml). We found higher median plasma concentrations of PFCs among cases compared with controls (Table 1). Women who did not plan their pregnancy were excluded from this analysis; however, based on women in our sample who were non-planners ($n=9$), mean levels of PFOS and PFOA were similar to planners (data not shown).

Crude ORs for subfecundity were higher among women with the highest PFOA or PFOS levels (Table 2). Although our data suggest a trend of increasing odds of subfecundity with increasing PFOS and PFOA levels, the strongest association for PFOA was in the third quartile. ORs for PFOA and PFOS were essentially unchanged after adjustment for maternal age at the pregnancy attempt, prepregnancy BMI, and maternal alcohol intake (PFOA analysis only).

After stratifying by parity, ORs for subfecundity were elevated only among parous women (Table 3). Among these women, the OR for the highest PFOS quartile was 2.1 (95% CI=1.2-3.8); among nulliparous women, the OR for the highest PFOS quartile was 0.7 (0.4-1.3). We found a similar pattern for PFOA. Among parous women, the OR for the highest quartile was 2.1 (1.0-4.4), while among nulliparous women, the OR for the highest quartile was 0.5 (0.2-1.2). Among parous women, there was evidence of a monotonic dose response for PFOS, and a stronger, but potentially non-linear, dose-response for PFOA, with the highest odds among women with PFOA levels again in the third quartile (2.4 [1.4-4.1]). Among nulliparous women, the ORs for both PFOS and PFOA appear to decrease with rising PFC levels, although these estimates are imprecise. In the adjusted models for parous women, the addition of a variable representing the interpregnancy interval minus time to pregnancy did not change the association between either type of PFC and subfecundity (data not shown). The addition of a variable representing duration of breastfeeding did not affect

the association between PFOS and subfecundity, but did attenuate the OR of subfecundity among women in the highest PFOA quartile (1.6 [0.7 – 3.8]).

To better enable a comparison of our results with those from the Danish cohort,¹⁶ we attempted to categorize PFC levels using the quartiles from their study. PFOS levels for 96% of women in our study and PFOA levels for 91% of our women fell in the lowest (referent) quartile of the Danish study. All remaining women in our study had PFC levels that fell in the second quartile of the Danish study. Therefore, we reanalyzed our data dichotomizing exposure at the cutpoint between the Danish referent and second quartile. Among the more highly exposed women in our study, there was no association between PFOS and subfecundity for either parous or nulliparous women. For PFOA, parous women had increased odds of subfecundity while nulliparous women had decreased odds of subfecundity (data not shown).

Among parous subjects, the median plasma PFOS concentration was 7% higher in cases than in controls, and the median PFOA concentration was 14% higher in cases than controls. With respect to the prediction of PFOS levels, the interpregnancy interval ($\beta=0.0030$ [standard error (SD)=0.0006]), but not breastfeeding duration ($\beta=0.0006$ [SD=0.0031]), was an important predictor of PFOS levels; for PFOA levels, both the interpregnancy interval ($\beta=0.0041$ [SD=0.0006]) and breastfeeding duration ($\beta=-0.0168$ [SD=0.0031]) were important predictors. The beta coefficient corresponding to the interpregnancy interval represents an increase in $\ln(\text{PFC})$ levels for each month since the previous birth. The beta coefficient corresponding to the breastfeeding variable for PFOA represents a decrease in $\ln(\text{PFC})$ levels for each month spent breastfeeding the previous child. By multiplying these coefficients by the difference between cases and controls in average time to pregnancy and in average breastfeeding duration, we estimated the percent of PFC levels that might be explained by differences in these factors. We estimated that differences in time to pregnancy accounted for 7% increased PFOS levels and 10% increased PFOA levels among cases compared with controls. We further estimated that differences in breastfeeding duration accounted for 4% higher levels of PFOA among cases. In total, this model estimated 7% higher levels of PFOS and 13% higher PFOA levels among cases, similar to the differences seen in our data.

Discussion

We observed increased ORs for subfecundity associated with PFOS and PFOA, although only among parous women. Among nulliparous women higher PFC plasma levels were associated with a decreased odds of subfecundity, suggesting that an unmeasured correlate of exposure is also related to fecundability. But the association was imprecise and, without replication by others, its importance is unclear. We also found that, among parous women, the interpregnancy interval and breastfeeding duration accounted for much of the difference in median PFOS and PFOA levels between cases and controls.

Data regarding reproductive toxicity of PFCs are limited and inconsistent. There have been reports of increased fetal resorptions in PFOS-exposed rabbits²³ and PFOA-exposed mice,²⁴ but a third study has found no alterations in fertility or litter size in rats exposed to ammonium perfluorooctanoate.²⁵ More importantly, the relevance of the results of these animal studies to human populations is questionable in that exposures in these studies were much higher than in background-exposed humans.

A study in the Danish National Birth Cohort¹⁶ found increased relative odds of subfecundity among women in the three highest quartiles of both PFOS and PFOA. In that study, estimates were adjusted for several variables (including parity, paternal age, paternal

education, and maternal socioeconomic status) not included in our final models. Rather than adjust for parity, we were interested in potential differences in effects of PFCs in parous and nulliparous women. We stratified by parity (defined by live births and losses after 20 weeks) rather than gravidity so as to capture only those pregnancies which would have the largest potential impact on PFC levels. (Our results were largely unchanged, however, when stratified instead by gravidity.) The evaluation of confounding in the present study included, among other variables, each of the covariates adjusted for in the Danish study, with the exception of maternal socioeconomic status (we included maternal education in its place). Based on this assessment, inclusion of other variables did not change our effect estimates for PFOS by more than 10%, and only maternal alcohol intake influenced the effect estimates for PFOA. The present study also used a sampling scheme whereby eligible women were sampled and included based on their reported time to pregnancy, ensuring a large number of cases for analysis. The study in the Danish cohort captured a random sample of all eligible women, resulting in fewer subfecund women.

There are other important differences between the Danish study and ours. The PFC levels observed in the Danish cohort were much higher than observed in the present study. Among women included in the Danish cohort, median exposure levels of PFOS and PFOA were more than double the levels observed in Norway. Fewer than 10% of the women in our study had PFC levels above the lowest quartile in Denmark. Further, the blood samples collected in the Danish cohort were obtained from 1996 through 2002. Studies document declining PFC levels over time; however, the decline likely did not begin until around 2000 and may be attributed to the phase-out of some PFCs. Two biomonitoring studies in the U.S. reported that blood levels of PFOS and PFOA had begun to decline by 2003-2004 (compared with 1999-2000 levels)²⁶ and 2006 (compared with 2000-2001 levels).²⁷ A study of archived human serum samples from Norway also showed a decline in PFOS and PFOA levels beginning around 2000.¹² In contrast, among Chinese women, serum levels of PFOS and PFOA increased from 1999 to 2002.²⁸ Over the seven-year period when blood samples were collected in the Danish cohort, levels of PFOS and PFOA may still have been increasing, which could affect results of analyses of subfecundity and PFCs. When PFC body burden among all women is increasing, those with longer time-to-pregnancy would tend to have higher PFC blood levels — even among nulliparous women — resulting in reverse causality.

In the present study, women were recruited early in pregnancy and asked to recall their time to pregnancy. The distribution of self-reported time to pregnancy in retrospective studies is reasonably accurate as many as 20 years later.^{29,30} Further, even if women in our study inaccurately reported their time to pregnancy, differential misclassification by exposure is unlikely because women were unaware of their PFC level. Because women were recruited for the Norwegian study during pregnancy, women who experienced miscarriage or who were sterile were not represented. The sample of women included in the present analysis was further restricted to live births. The exclusion of sterile women and those with pregnancy losses limits the generalizability of our results; also, their exclusion may result in underestimation of a true effect of PFCs on subfecundity.

Unexpectedly, we found a larger proportion of cases aged less than 25 years at the time of the pregnancy attempt compared with controls. After further inspection, we found that approximately 44% of these women had received fertility treatment for the index pregnancy (a proportion similar to women in the next age group), providing evidence that these women are truly cases. Further, our study represents a relatively small sample of women from the larger Norwegian study of over 90,000 women. The increase in the proportion of very young cases was likely due to a random selection process that, by chance, generated a younger case group. Excluding the youngest age group from the analysis did not change our results.

Because the present study included a large number of subfecund women, we were able to study the effects of PFCs on subfecundity by parity. On the other hand, the simple linear regression model used in our sensitivity analysis to estimate expected differences in PFC concentrations between cases and controls may be inaccurate, as there could be other factors besides just the two we modeled that affect PFC levels during early pregnancy.

The discrepant results we observed among parous and nulliparous women may be explained by factors related to pregnancy history. As noted earlier, there is a complex relation between a woman's pregnancy history and current levels of environmental toxicants, particularly when exposures to the toxicant vary over time.¹⁸ Due to the pharmacokinetics of PFCs during pregnancy and lactation, an apparent association between PFCs and subfecundity may be produced even when a causal association does not exist. It is possible that following the decline in maternal PFC levels observed during pregnancy, delivery, and lactation, levels again rise to baseline. Therefore, as mentioned earlier, a long interval between the birth of the previous child and the start of the next pregnancy attempt will allow for a longer time during which levels can rise – potentially resulting in a non-causal association between subfecundity and PFC levels. Results from women with no previous pregnancies may be more informative regarding toxic effects of these compounds. Based on the nulliparous women in our study, we found no evidence of an adverse effect on subfecundity at the PFC levels in our population.

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References

1. Organisation for Economic Co-operation and Development (OECD). Series on Risk Management. ENV/JM/MONO(2005)1. Paris: 2005. Results of survey on production and use of PFOS, PFAS and PFOA, related substances and products/mixtures containing these substances.
<http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282005%291&docLanguage=En>
2. Kantiani L, Llorca M, Sanchís J, Farré M, Barceló D. Emerging food contaminants: a review. *Analytical and Bioanalytical Chemistry*. 2010;1–15.
3. Fromme H, Tittlemier SA, Völkel W, Wilhelm M, Twardella D. Perfluorinated compounds - Exposure assessment for the general population in western countries. *International Journal of Hygiene and Environmental Health*. 2009; 212(3):239–270. [PubMed: 18565792]
4. Giesy JP, Kannan K. Perfluorochemical surfactants in the environment. *Environ Sci Technol*. 2002; 36(7):146A–152A. [PubMed: 11827047]
5. Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, Faber F, Hannibal I, Genzel-Boroviczeny O, Koletzko B, Völkel W. Pre- and Postnatal Exposure to Perfluorinated Compounds (PFCs). *Environmental Science and Technology*. 2010; 44(18):7123–7129. [PubMed: 20722423]
6. Kato K, Calafat AM, Wong LY, Wanigatunga AA, Caudill SP, Needham LL. Polyfluoroalkyl Compounds in Pooled Sera from Children Participating in the National Health and Nutrition Examination Survey 2001–2002. *Environmental Science & Technology*. 2009; 43(7):2641–2647. [PubMed: 19452929]
7. Llorca M, Farré M, Pico Y, Teijon ML, Álvarez JG, Barceló D. Infant exposure of perfluorinated compounds: Levels in breast milk and commercial baby food. *Environment International*. 2010; 36(6):584–592. [PubMed: 20494442]

8. Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environmental Health Perspectives*. 2007; 115(11):1670–1676. [PubMed: 18008002]
9. Fei C, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environmental Health Perspectives*. 2007; 115(11):1677–1682. [PubMed: 18008003]
10. Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, Zobel LR. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environmental Health Perspectives*. 2007; 115(9):1298–1305. [PubMed: 17805419]
11. United States Environmental Protection Agency (EPA). [Accessed: January 15 2011] 2010/2015 PFOA Stewardship Program. 2010. Available: <http://www.epa.gov/opptintr/pfoa/pubs/stewardship/>
12. Haug LS, Thomsen C, Becher G. Time Trends and the Influence of Age and Gender on Serum Concentrations of Perfluorinated Compounds in Archived Human Samples. *Environmental Science & Technology*. 2009; 43(6):2131–2136. [PubMed: 19368225]
13. Sundstrom M, Ehresman DJ, Bignert A, Butenhoff JL, Olsen GW, Chang SC, Bergman A. A temporal trend study (1972–2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. *Environ Int*. 2011; 37(1):178–83. [PubMed: 20880590]
14. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: A review of monitoring and toxicological findings. *Toxicological Sciences*. 2007; 99(2):366–394. [PubMed: 17519394]
15. Kennedy GL Jr. Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG. The toxicology of perfluorooctanoate. *Crit Rev Toxicol*. 2004; 34(4):351–84. [PubMed: 15328768]
16. Fei CY, McLaughlin JK, Lipworth L, Olsen J. Maternal levels of perfluorinated chemicals and subfecundity. *Human Reproduction*. 2009; 24(5):1200–1205. [PubMed: 19176540]
17. Thomsen C, Haug LS, Stigum H, Frøshaug M, Broadwell SL, Becher G. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. *Environmental Science and Technology*. 2010; 44(24):9550–9556. [PubMed: 21090747]
18. Olsen GW, Butenhoff JL, Zobel LR. Perfluoroalkyl chemicals and human fetal development: An epidemiologic review with clinical and toxicological perspectives. *Reproductive Toxicology*. 2009; 27(3-4):212–230. [PubMed: 19429401]
19. Magnus P, Irgens LM, Haug K, Nystad W, Skjærven R, Stoltenberg C. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol*. 2006; 35(5):1146–50. [PubMed: 16926217]
20. Frömel T, Knepper TP. Biodegradation of fluorinated alkyl substances. *Reviews of Environmental Contamination and Toxicology*. 2010; Vol. 208:161–177.
21. Haug LS, Thomsen C, Becher G. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. *Journal of Chromatography A*. 2009; 1216(3):385–393. [PubMed: 19026423]
22. Rothman, KJ.; Greenland, S. *Modern Epidemiology*. 2 ed. Lippincott Williams & Wilkins; Philadelphia, PA: 1998.
23. Case MT, York RG, Christian MS. Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *International Journal of Toxicology*. 2001; 20(2):101–109. [PubMed: 11354466]
24. Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, Strynar MJ. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicological Sciences*. 2006; 90(2):510–518. [PubMed: 16415327]

25. Butenhoff JL, Ehresman DJ, Chang SC, Parker GA, Stump DG. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K^+PFOS) in rats: Developmental neurotoxicity. *Reproductive Toxicology*. 2009; 27(3-4):319–330. [PubMed: 19162172]
26. Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the US population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environmental Health Perspectives*. 2007; 115(11):1596–1602. [PubMed: 18007991]
27. Olsen GW, Mair DC, Church TR, Ellefson ME, Reagen WK, Boyd TM, Herron RM, Medhdizadehkashi Z, Nobiletti JB, Rios JA, Butenhoff JL, Zobel LR. Decline in perfluorooctanesulfonate and other polyfluoroalkyl chemicals in American red cross adult blood donors, 2000-2006. *Environmental Science and Technology*. 2008; 42(13):4989–4995. [PubMed: 18678038]
28. Jin YH, Saito N, Harada KH, Inoue K, Koizumi A. Historical trends in human serum levels of perfluorooctanoate and perfluorooctane sulfonate in Shenyang, China. *Tohoku Journal of Experimental Medicine*. 2007; 212(1):63–70. [PubMed: 17464105]
29. Joffe M. Validity of self-reported time to pregnancy. *Epidemiology*. 2010; 21(1):160–161. [PubMed: 20010224]
30. Joffe M, Villard L, Li Z, Plowman R, Vessey M. A time to pregnancy questionnaire designed for long term recall: Validity in Oxford, England. *Journal of Epidemiology and Community Health*. 1995; 49(3):314–319. [PubMed: 7629471]

Table 1

Characteristics of 910 study subjects from the Norwegian Mother and Child Cohort Study, Norway, 2003-2004

	Cases (N = 416)	Controls (N = 494)
	%	%
Age at Pregnancy Attempt (years)		
<25	21	14
25-29	41	42
30-34	29	34
≥35	9	10
Prepregnancy BMI (kg/m ²)		
<18.50	3	2
18.50-24.99	52	63
25.00-29.99	28	25
≥30.00	16	10
Smoking before pregnancy		
Daily	24	17
Sometimes	11	10
None	66	74
Maternal alcohol use 3-months before pregnancy		
1+ time/week	18	26
1-3 times/month	31	34
<1 time/month and never	51	39
Missing	1	1
Maternal Education		
< High School	11	7
High School	34	30
Some College	39	44
4+ Years of College	16	19
Missing	<1	<1
Gravidity		
0	41	31
1+	59	69
Parity		
0	59	41
1	29	41
2+	12	18
Interval between previous birth and pregnancy attempt of index child (months); mean (SD) ^a	38 (37)	38 (32)
Duration of breastfeeding previous born child (months); mean (SD) ^b	7 (6)	9 (6)
PFOS, (ng/ml); median (IQR)	14 (10.7-16.9)	13 (10.1-16.2)

	Cases (N = 416)	Controls (N = 494)
	%	%
PFOA, (ng/ml); median (IQR)	2 (1.8-3.1)	2 (1.5-2.9)

^a restricted to parous women, (169 cases and 293 controls); 6 cases missing interval information

^b restricted to parous women, (169 cases and 293 controls); 6 cases and 8 controls missing breastfeeding information

Association between PFCs and subfecundity among 9104 subjects from the Norwegian Mother and Child Cohort Study, Norway, 2003-2004

Table 2

	No. Cases	No. Controls	Crude		Adjusted ^b	
			OR	(95% CI)	OR	(95% CI)
PFOS (ng/ml)						
<10.34 ^c	90	140	1.0		1.0	
10.34 - 13.09	104	124	1.3	(0.9-1.9)	1.3	(0.9-1.9)
13.10 - 16.60	110	118	1.5	(1.0-2.1)	1.4	(1.0-2.0)
≥16.61	112	112	1.6	(1.1-2.3)	1.6	(1.1-2.3)
Test for trend ^d				P=0.02		P=0.02
PFOA (ng/ml)						
<1.66 ^c	77	146	1.0		1.0	
1.66 - 2.24	104	122	1.6	(1.1-2.4)	1.6	(1.1-2.3)
2.25 - 3.02	118	105	2.1	(1.5-3.1)	2.2	(1.5-3.2)
≥3.03	113	115	1.9	(1.3-2.7)	2.0	(1.4-3.0)
Test for trend ^d				P<0.01		P<0.001

^a PFOA analysis has 900 subjects due to missing covariate information

^b PFOS model adjusted for maternal age at pregnancy attempt and prepregnancy BMI; PFOA model additionally adjusted for maternal drinking before pregnancy

^c Reference category

^d p-values were calculated using categories representing the median value of the corresponding quartile

Association between PFCs and subfecundity among 910^a subjects in the Norwegian Mother and Child Cohort Study, Norway, 2003-2004, stratified by parity

Table 3

	Primiparous			Parous		
	No. Cases	No. Controls	Adjusted ^b OR (95% CI)	Cases No.	Controls No.	Adjusted ^b OR (95% CI)
PFOS (ng/ml)						
<10.34 ^c	45	32	1.0	45	108	1.0
10.34 - 13.09	55	42	0.8 (0.4-1.6)	49	82	1.5 (0.9-2.5)
13.10 - 16.60	73	60	0.8 (0.4-1.4)	37	58	1.5 (0.9-2.6)
≥16.61	78	72	0.7 (0.4-1.3)	34	40	2.1 (1.2-3.8)
Test for trend ^d			<i>P</i> =0.30			<i>P</i> =0.01
PFOA (ng/ml)						
<1.66 ^c	23	13	1.0	54	133	1.0
1.66 - 2.24	52	37	0.6 (0.3-1.5)	52	85	1.5 (0.9-2.5)
2.25 - 3.02	78	59	0.6 (0.3-1.4)	40	46	2.4 (1.4-4.1)
≥3.03	95	94	0.5 (0.2-1.2)	18	21	2.1 (1.0-4.4)
Test for trend ^d			<i>P</i> =0.20			<i>P</i> =0.01

^a PFOA analysis has 900 subjects due to missing covariate information

^b PFOS model adjusted for maternal age at pregnancy attempt and prepregnancy BMI; PFOA model additionally adjusted for maternal drinking before pregnancy

^c Reference category

^d p-values were calculated using categories representing the median value of the corresponding quartile

note: p-value for interaction between PFOS (ng/ml) and parity (0 vs. 1+) = 0.04; p-value for interaction between PFOA (ng/ml) and parity (0 vs. 1+) < 0.001