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Biomonitoring PFAAs in blood and semen samples: Investigation of a potential link between PFAAs exposure and semen mobility in China

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ABSTRACT

Perfluoroalkyl acids (PFAAs) have been suspected to act as endocrine disruptors and adversely affect human reproductive health. We aimed to investigate the association between PFAAs in blood and semen, explore a potential link between PFAAs exposure and semen quality in the population of the Pearl River Delta (PRD) region in China, one of the "world factories". The monitoring results demonstrated that the population (103 male participants) from the PRD region in this study had higher PFAAs levels in blood and semen than some other areas in China. PFOS was found at the highest mean concentrations of 118.16 ng/mL in blood and 5.31 ng/mL in semen among the nine PFAAs. Significant associations were found between concentrations of several analytes in blood and semen, including Σ_9 PFAAs ($r = 0.475$, $P < .01$), PFOA ($r = 0.215$, $P = .029$), PFHS ($r = 0.458$, $P < .01$) and PFOS ($r = 0.981$, $P < .01$). BMI was the most important factor to PFAAs, but there was no significant difference in PFAAs concentrations in blood and semen collected from participants with different smoking and drinking habits, education background and occupations. Negative correlations were significantly observed between sperm motility and PFBA, PFPeA, PFHxA, PFBS, PFOA, PFHS, PFOS and Σ_9PFAAs in semen. Therefore, exposure to PFAAs may result in a decline in semen mobility in participants from the PRD region.

1. Introduction

Perfluoroalkyl acids (PFAAs) are synthetic chemicals with hydrophobic and lipophobic properties. The strong carbon-fluorine covalent bonds in PFAAs account for thermal and chemical stability, which have led to a wide use of these chemicals for industrial purposes and consumer products such as carpets, paper, extinguisher foams, clothing and non-stick cookware (Prevedouros et al., 2006; Hekster et al., 2003). In China, PFOS and PFOA have been banned in products since 2014 (Yang et al., 2014). The most prevalent PFAAs include perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), which are also suspected of carcinogenic activity and hormonal disruption (Jensen and Leffers, 2008; Kennedy et al., 2004).

PFAAs are ubiquitous in air, water, and soil worldwide (Annamalai and Namasivayam, 2015; Duong et al., 2015; Ellis et al., 2004; Plassmann and Berger, 2013; Yang et al., 2011; Zhao et al., 2013), and have also been detected in human blood, breast milk, urine, hair, and tissues (Zhao et al., 2013; Yeung et al., 2009; Mosch et al., 2010; Kim et al., 2014b; Li et al., 2012; Maestri et al., 2006; Rotander et al., 2015). In a US study consisting of 256 participants, the concentration of PFOS and PFOA in plasma was 32.3 and 9.2 ng/mL, respectively (Raymer et al., 2012). In China, a similar concentration (14.01 ng/mL) of PFAAs (sum of PFOS and PFOA) was found in blood samples from 141 participants in Tangshan (Guo et al., 2011). Lu et al. (2014) found higher concentrations of PFOS and PFOA in blood from textile workers (5.73 and 5.46 μg/L, respectively), who may face higher occupational exposure to PFAAs than a control group (2.55 and 2.84 μg/L in barbers) since PFAAs are widely used in fabrics, carpets, and other textile products. Numerous investigations have been conducted on the exposure of PFAAs in humans around the world, and concern about the potential health consequences of PFAAs has been raised, especially regarding male reproductive health (Genuis et al., 2010; Joensen et al., 2009; Vested et al., 2013).

Semen can be also used for biomonitoring studies (Esteban and

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Castaño, 2009), but it has only been used rarely for PFAAs. In a US study consisting of 256 participants, the concentration of PFOS was 0.6 ng/mL in semen, and only 2% of the semen PFOA concentrations were reported greater than the limit of quantitation (Raymer et al., 2012).

Exposure to environmental endocrine disruptors may contribute to the observed decline in male semen quality (Hauser et al., 2005; Swan et al., 2003; Xia et al., 2008). However, studies investigating the association between PFAAs at a low level and semen quality are limited, and the results have been inconsistent. A study in Denmark, Joensen et al., 2012 concluded that high PFAAs levels in serum of 247 healthy men from the general population were not significantly associated with semen quality. Similarly, there was no indication that PFOA or PFOS were significantly associated with any semen quality parameters (semen volume, sperm concentration, percent motility, swim-up motility and concentration, and directional motility) in Raymer et al., 2012. However, the study by Vested et al. (2013) suggested that in utero exposure to PFOA was associated with lower adjusted sperm concentration and total sperm count. Moreover, PFAAs at environmentally relevant concentrations were associated with differences in sperm head, morphology, and DNA characteristics, including differences indicative of higher and lower semen quality in Louis et al., 2015.

Although several studies have detected PFAAs in blood, and explored the association between PFAAs and semen quality, the relationships are weak in most cases. One possible reason is that some other factors (i.e. PFAAs concentrations in semen) are more important to semen quality than PFAAs concentrations in blood. In this study, we aim to investigate the association between PFAAs in blood/semen and their effects on semen quality, with an emphasis on semen mobility, in the general population in the Pearl River Delta (PRD) region, China. This could give us a better understanding on how PFAAs affect semen quality.

2. Materials and methods

2.1. Studied population and sample collection

Samples collection was undertaken in the infertility clinic at the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou city, Guangdong province, China, during July 2012 to August 2013, using the methodology described previously (Song et al., 2013). Patients with genital damage, venereal disease or azoospermia were excluded from the study. In total, 103 male participants were finally involved in this study. Blood samples were drawn from phlebotomizing. Semen samples were collected by masturbation into a sterile wide polypropylene container after at least 2–7 days of sexual abstinence. All samples were stored at −80 °C in polypropylene tubes for PFAAs. Informed consent was obtained from all participants prior to conducting the study, and the study was approved by the Third Affiliated Hospital of Sun Yat-sen University Ethics Committee (Approve number: 2013-2-72).

2.2. Questionnaire recruitment

All participants were recruited to answer a questionnaire including basic information (age, weight and height), educational background and living habits (drinking and smoking).

2.3. Materials

The providers of PFAAs native and internal standards are shown in Table S1 (Supplemental material). Liquid chromatography grade *n*-hexane and MeOH were purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q system (Merck Millipore, MA, USA). Dionex OnGuard™ II H, OnGuard™ II Ag, and OnGuard™ II Ba cartridges were purchased from Thermo Fisher (Bremen, Germany). Hydromatrix was purchased from Agilent Technologies (Santa Clara, CA, USA), and alumina (150 mesh) was purchased from Sigma Aldrich (St Louis, MO, USA). Diatomaceous earth (Damolin AS, Denmark) was previously baked at 450 °C for 12 h in this study.

2.4. Analytical method

Spiked with 10 ng internal standards, blood and semen samples were dried by a freeze dryer (VirTis 4KBTXL-75, USA) for 48 h. Samples were weighed before and after freeze drying. The dried samples were extracted by an ASE-SPE system. Sorbents were packed into 33 mL ASE cells, including, from bottom to top, glass fiber filter, 3.0 g OnGuard H, 6.0 g OnGuard Ag, 3.0 g OnGuard Barium, 18 g basic Alumina, 1.8 g OnGuard RP, hydromatrix. Samples were then extracted using methanol, and a mixture of *n*-Hexane and MeOH (1:1, v:v) for 1 and 3 static cycles, respectively. The other parameters were: temperature 100 °C, pressure 1500 psi, static time of 5 min per cycle, flush volume 60% and purge time 60 s. Both fractions were combined and concentrated, and then suspended by 500 μL MeOH.

2.5. Instrumental analysis

An Agilent 1200 series HPLC equipped with an Agilent 6410 QQQ mass spectrum system was used for analysis. Separation was achieved using an Agilent ZORBAX Eclipse Plus C18 column (2.1 × 100 mm id. 1.8 μm particle size), operated at a temperature of 40 °C. Mobile phase composition was A: 2 mM ammonium acetate solution in water, and B: 2 mM ammonium acetate solution in acetonitrile. A linear gradient profile was used, starting at 40% B, ramped to 90% B in 9 min, then held at 90% B for 4 min. MS was operated in the negative ESI mode, using multiple reaction mode (MRM). The optimized instrument settings were: Source temperature 350 °C, desolvation temperature: 350 °C, desolvation (curtain) gas (N_2) flow: 12 L/h, nebulizer gas (N_2) flow: 9 L/h, pressure: 35 psi, Capillary: −4000 V. Compound MRM parameters are shown in Table S2 (Supplemental material).

2.6. Quality control/quality assurance

The instruments were calibrated daily. A procedural blank, a spiked blank, a matrix spike, and a sample duplicate were processed for each batch of 5 samples. None of the target PFAAs was detected in procedural blanks. The regression coefficients (r^2) of calibration curves for target PFAAs were higher than 0.999. Limits of detection (LOD) were calculated as the average concentration of procedural blanks plus three times the standard deviation (SD) of the blanks, respectively. For analytes not present in the blanks, LODs were calculated from signal/noise ratio of 10. Sample results were not corrected for the surrogate recoveries or blank values. The linear range, LOD, LOQ, RSD and average recovery for the target PFAAs are summarized in Table S3 (Supplemental material).

2.7. Semen investigation

Semen quality analysis was performed in a semen analysis laboratory in the infertility department at the same hospital. Samples were analyzed using the conventional method of the World Health Organization (Cooper et al., 2010). As suggested by Cooper (Cooper et al., 2010), total motility (sum of grade a, b, and c) and progressive motility (sum of grade a and b) were measured in this study. Semen concentrations were determined using 100-μm-deep hemacytometer chambers by observation with microscopy.

2.8. Statistical analysis

Statistical analyses were performed by using SPSS 19.0 23 (Chicago, USA) software package for Windows. Spearman correlations were used to examine correlations among PFAAs concentration, semen parameters,