Science of the Total Environment 605–606 (2017) 9–17

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Associations between perfluorinated alkyl acids in blood and ovarian follicular fluid and ovarian function in women undergoing assisted reproductive treatment

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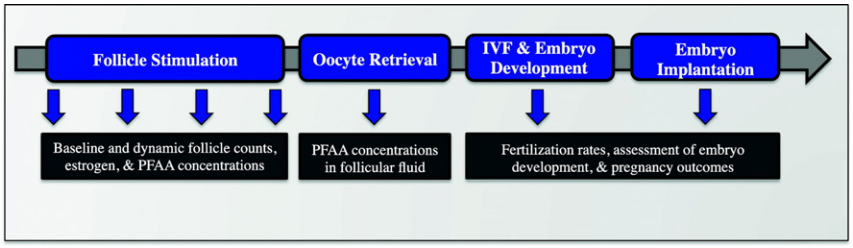
HIGHLIGHTS

- Perfluorinated alkyl acids (PFAAs) are investigated to determine associations with ovarian responsiveness and fertility.
- Assessments of ovarian follicular fluid indicate measurable levels of six PFAA compounds.
- PFAA concentrations in blood are highly correlated to those in ovarian follicular fluid.
- PFAA concentrations negatively correlate to certain fertility outcomes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 May 2017
Received in revised form 15 June 2017
Accepted 17 June 2017
Available online 23 June 2017

Editor: D. Barcelo

Keywords:
PFAAs
EDCs
Follicular fluid
ART

ABSTRACT

Endocrine disrupting contaminants, in combination with other environmental variables, are associated with altered reproductive health. Assisted reproductive technology (ART) procedures offer valuable opportunities to explore the connections between environmental contaminants in the ovarian microenvironment and measures of fertility, including impaired responsiveness to gonadotropins. Here, we investigate an emerging class of environmental contaminants, the perfluorinated alkyl acids (PFAAs), to determine whether ovarian contaminant levels are associated with measures of ovarian responsiveness and fertility outcomes in a South Carolina population of women undergoing ART. Levels of PFAAs in plasma and follicular fluid samples collected from women undergoing ovarian stimulation were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Six PFAAs were detected in both plasma and follicular fluid. PFAA concentrations in plasma correlate strongly to those detected in ovary and, with the exception of one compound, remain stable throughout ovarian stimulation. The concentration of PFHxS in follicular fluid inversely relates to baseline follicle counts. While no significant relationships were detected between ovarian response measures and PFAA concentrations, we identified a negative relationship between follicular fluid PFDA and PFuNA and blastocyst conversion rates. Our assessments

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http://dx.doi.org/10.1016/j.scitotenv.2017.06.137

indicate that plasma levels of PFAAs serve as a sound proxy of those in the ovarian compartment and that follicular fluid levels of specific PFAA compounds are inversely related to important clinical measures of reproductive health including baseline follicle count and post-fertilization success.

1. Introduction

Current estimates of human fecundity and fertility in the United States indicate that 10.9% (7.3 million) of married women between ages 15–44 display impaired fecundity and 6% (1.5 million) are classified as infertile (Chandra et al., 2013; Chandra et al., 2005). While it is difficult to evaluate whether these numbers have increased over recent decades, it is clear that the number of individuals seeking assisted reproductive technology (ART) therapy is on the rise (Boivin et al., 2007). Various reasons have been postulated to explain this trend including delayed age of first pregnancy, improved access to fertility clinics, and increased reporting of fertility problems. Additionally, exposure to environmental contaminants can significantly influence reproductive health, and exposures have been suggested as a likely contributor (Younglai et al., 2002; Bloom et al., 2010; Bernanke and Kohler, 2009; Herbst and Bern, 1981). Here, we investigate an emerging class of environmental contaminants, the perfluorinated alkyl acids (PFAAs), to determine whether ovarian contaminant levels are associated with measures of overall ovarian health in a population of women undergoing fertility treatment.

The ovarian follicle represents the functional unit of the ovary, and follicular development is tightly regulated by both intra- and extra-ovarian factors (Edson et al., 2009). Whereas pre-antral stages can occur independent of gonadotropin receptor activation and are characterized by growth and differentiation of the oocyte, the subsequent antral phases of follicular development are dependent upon pituitary derived gonadotropins (follicle stimulating hormone and luteinizing hormone, FSH and LH, respectively) and are characterized by differentiation and enlargement of the follicle (Edson et al., 2009). Thus, the ability of the ovary to properly respond to gonadotropin stimulation is a fundamental aspect of its function. Current ART takes advantage of the regulatory mechanisms that control follicle growth to promote the development of multiple follicles. In this setting, controlled ovarian stimulation is conducted by pharmacologically silencing the hypothalamus-pituitary-ovary axis and subsequent stimulation with exogenous FSH, allowing for careful control and monitoring of the developing follicle pool. When optimal follicle count and size is achieved, ovulation is triggered and measures to promote fertilization (*e.g.*, intrauterine insemination, harvesting of follicles subsequent *in vitro* fertilization) are performed (Yang et al., 2013). Ovarian responsiveness to FSH treatment is not a standardized parameter across different fertility practices, but typically incorporates a combination of more common measures including peak 17β-estradiol (E_2), peak follicle count, total FSH administered, and total oocytes retrieved. The etiology of variation in responsiveness to FSH is likely rooted in a complex of influences including age, body mass index (BMI), ovarian reserve, as well as genetic factors. There is, however, also concern that exposures to environmental contaminants may also impair the mechanisms that regulate ovarian follicle development (Guillette and Moore, 2006; Borgeest et al., 2002; Gupta et al., 2009). The treatment protocol and samples collected as part of standard ART procedures offer valuable opportunities to explore the connections between environmental contaminants in the ovarian microenvironment potentially providing insights into the underlying influences of impaired responsiveness to gonadotropins.

Here we focus on PFAAs, which exhibit surfactant properties that make them desirable components of various products including adhesives, water-repellant surfaces, lubricants, and aqueous film-forming foams finding use in packaging, as stain repellants on fabrics and as firefighting foams. Structurally, PFAAs consist of one or more carbon atoms where all of the hydrogen atoms have been replaced by fluorine atoms and an acid functional group (Buck et al., 2011). The carbon-fluorine bond is the strongest known bond in organic chemistry hence PFAAs are stable and long-lived in the environment. PFAAs have also been shown to bioaccumulate and biomagnify in the ecosystem (Houde et al., 2011; Rotander et al., 2012). In humans, PFAAs are poorly eliminated and exhibit half-lives up to 5 years (Olsen et al., 2007). Primary exposure routes include inhalation of air particles contaminated with PFAAs originating from numerous consumer products including non-stick cookware and water-resistant consumables as well as ingestion through food and water (D'Hollander et al., 2010). Some epidemiological data exists associating PFAAs and health outcomes including recent studies demonstrating an association between circulating PFAA levels and kidney dysfunction (increased uric acid, reduced glomerular filtration), prostate cancer risk, lipid metabolism, and sperm quality (Hardell et al., 2014; Kataria et al., 2015; Fletcher et al., 2013; Vested et al., 2013).

Both epidemiological and *in vitro* studies suggest that PFAA compounds might influence ovarian cell signaling and measures of overall reproductive health (Crawford et al., 2017). In human populations, elevated concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are associated with moderate to severe endometriosis as well as polycystic ovarian syndrome (Vagi et al., 2014; Louis et al., 2012). Approaches that incorporate transient transfection assays have linked PFAA exposure to activation of mouse and human peroxisome proliferator activated receptor alpha (PPARα) (Takacs and Abbott, 2007; Wolf et al., 2012). PPARs are ligand dependent nuclear receptors with roles in many physiological processes including inflammation, energy homeostasis, glucose metabolism and cellular proliferation and differentiation. The observed interaction between PFAAs and receptors of the PPAR family is intriguing given the documented role of PPAR receptor activation in the regulation of folliculogenesis and steroidogenesis and offers rationale for inclusion of the PFAA survey conducted here (Velez et al., 2013; Froment et al., 2006). To our knowledge, only two studies have directly assessed the relationship between PFAAs and ovarian health, specifically responsiveness to gonadotropin stimulation through fertility treatment. The first did not present details regarding individual PFAA concentrations (Governini et al., 2011). A more recent study involved assessments in a population of Belgium women seeking ART and focused on examining post-oocyte retrieval outcomes such as fertilization and cleavage rates and identified a direct relationship between PFAA concentrations and embryo quality (Petro et al., 2014).

The development of the ovarian follicle is a tightly regulated process dependent on a critical balance of hormones and growth factors. Given the observations linking PFAAs to disrupted ovarian signaling, we set out to address four primary questions (1) Which PFAAs are measurable in the ovarian follicular fluid?, (2) Are plasma PFAA levels predictive of levels measured in follicular fluid?, (3) Do the concentrations of PFAAs fluctuate over the course of ovarian stimulation with FSH?, and (4) Is there relationship between measures of ovarian responsiveness or fertilization success and PFAA body burden? The results will provide information regarding abundance and distribution of these compounds in a population of women seeking ART. Also important is that the analysis framework described here can be applied to study the influence of other compounds of interest on ovarian function and responsiveness in the setting of ART.