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Paternal urinary concentrations of organophosphate flame retardant metabolites, fertility measures, and pregnancy outcomes among couples undergoing in vitro fertilization

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ABSTRACT

Background: Use of organophosphate flame retardants (PFRs) has increased over the past decade following the phase out of some brominated flame retardants, leading to increased human exposure. We recently reported that increasing maternal PFR exposure is associated with poorer pregnancy outcomes among women from a fertility clinic. Because a small epidemiologic study previously reported an inverse association between male PFR exposures and sperm motility, we sought to examine associations of paternal urinary concentrations of PFR metabolites and their partner's pregnancy outcomes.

Methods: This analysis included 201 couples enrolled in the Environment and Reproductive Health (EARTH) prospective cohort study (2005–2015) who provided one or two urine samples per IVF cycle. In both the male and female partner, we measured five urinary PFR metabolites [bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), *tert*-butylphenyl phenyl phosphate (tb-PPP) and bis(1-chloro-2-propyl) phosphate (BCIPP)] using negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS). The sum of the molar concentrations of the urinary PFR metabolites was calculated. We used multivariable generalized linear mixed models to evaluate the association of urinary concentrations of paternal PFR metabolites with IVF outcomes, accounting for multiple in vitro fertilization (IVF) cycles per couple. Models were adjusted for year of IVF treatment cycle, primary infertility diagnosis, and maternal urinary PFR metabolites as well as paternal and maternal age, body mass index, and race/ethnicity.

Results: Detection rates were high for paternal urinary concentrations of BDCIPP (84%), DPHP (87%) and ip-PPP (76%) but low for tb-PPP (12%) and zero for BCIPP (0%). We observed a significant 12% decline in the proportion of fertilized oocytes from the first to second quartile of male urinary ΣPFR and a 47% decline in the number of best quality embryos from the first to third quartile of male urinary BDCIPP in our adjusted models. An 8% decline in fertilization was observed for the highest compared to lowest quartile of urinary BDCIPP concentrations (95% CI: 0.01, 0.12, p-trend = 0.06).

Conclusions: Using IVF as a model to investigate human reproduction and pregnancy outcomes, we found that

Abbreviations: ART, assisted reproductive technologies; BCIPP, bis(1-chloro-2-propyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; BMI, body mass index; CI, confidence interval; DPHP, diphenyl phosphate; EARTH, Environment and Reproductive Health Study; GM, geometric mean; GnRH, gonadotropin releasing hormone; ICC, intraclass correlation coefficients; ip-PPP, isopropylphenyl phenyl phosphate; IQR, interquartile range; IVF, in vitro fertilization; LC-MS/MS, liquid chromatography tandem mass spectrometry; MDL, method detection limits; MGH, Massachusetts General Hospital; Mono-ITP, mono-substituted isopropyl triphenyl phosphate; PFR, organophosphate flame retardant; SG, specific gravity; SRM, standard reference material; tb-PPP, *tert*-butylphenyl phenyl phosphate; TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; TPHP, triphenyl phosphate

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paternal urinary concentrations of BDCIPP were associated with reduced fertilization. In contrast to previously reported findings for the female partners, the paternal urinary PFR metabolites were not associated with the proportion of cycles resulting in successful implantation, clinical pregnancy, and live birth. These results indicate that paternal preconception exposure to TDCIPP may adversely impact successful oocyte fertilization, whereas female preconception exposure to ΣPFRs may be more relevant to adverse pregnancy outcomes.

1. Introduction

Use of organophosphate flame retardants (PFRs) has increased over the past decade in the polyurethane foam of upholstered furniture with the phase out PentaBDE (Stapleton et al., 2009). PFRs are not chemically bonded to foam and have been shown to migrate into the air and dust of indoor environments (van der Veen and de Boer, 2012). This has led to ubiquitous human exposure, with PFR urinary metabolites detected in 90 to 100% of adult urine samples (Butt et al., 2014; Butt et al., 2016; Carignan et al., 2013a; Cequier et al., 2015; Hammel et al., 2016; Hoffman et al., 2014; Meeker et al., 2013a; Van den Eede et al., 2015).

We previously reported that maternal exposure to PFRs may adversely impact female reproductive health and pregnancy outcomes, as evidenced by strong inverse associations for the sum of three PFR metabolites with decreased proportions of fertilization, implantation, clinical pregnancy and live birth among women recruited from an academic fertility clinic (Carignan et al., 2017). This finding was consistent with animal studies, which have shown adverse effects on reproductive outcomes including decreased egg production, promotion of oocyte maturation, egg quality, hatching and survival among zebrafish and delayed hatching among chicken embryos (Farhat et al., 2013; Liu et al., 2013; Wang et al., 2015b; Wang et al., 2013). Experimental studies have also reported impacts of PFRs on male fecundity. These include a study of zebrafish exposed to tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) that reported decreased spermiation and a study of leydig cell tumor cells exposed to seven common PFRs that found adverse effects on leydig cell mitochondrial activity, cell survival, and superoxide production to a greater extent than the brominated flame retardants they have replaced (Schang et al., 2016; Wang et al., 2015b). In a small epidemiologic study on PFRs and male reproductive health among men recruited from a fertility clinic, there was a suggestive inverse association of sperm concentrations and motility with urinary metabolites of TDCIPP and triphenyl phosphate (TPHP) (Meeker et al., 2013b).

Although our earlier study showed associations of maternal urinary concentrations of PFRs with poorer pregnancy outcomes (Carignan et al., 2017), the paternal contribution to these outcomes needs to be considered given the potential for correlated exposures among couples and the limited data showing associations with poorer semen quality. Therefore we explored associations of *paternal* urinary concentrations of PFR metabolites and pregnancy outcomes among couples in a prospective cohort study, the Environment and Reproductive Health Study (EARTH) Study, using assisted reproductive technologies (ART) as a model to early developmental endpoints and pregnancy outcomes.

2. Methods

2.1. Participants

Study participants included male and female partners recruited into the EARTH study between 2005 and 2015 to evaluate environmental and dietary determinants of fertility among patients from Massachusetts General Hospital (MGH) Fertility Center. Age requirements for participation were 18–55 for men and 18–45 for women. The EARTH study was approved by the Human Studies Institutional Review Boards of the MGH and Harvard T.H. Chan School of Public Health. Participants signed an informed consent after the study procedures were explained by trained study staff and any questions were answered. A staff-administered questionnaire was used to collect demographic information from each participant including race/ethnicity, smoking history, education, and history of previous pregnancies. To be included in the present analysis, couples must have used their own fresh gametes and each partner must have provided at least one urine sample for the measurement of flame retardant metabolites during an in vitro fertilization (IVF) cycle. We included up to three IVF attempts per couple. Our final dataset included 201 couples with 276 IVF cycles who had complete information on the exposure and outcome variables.

2.2. Clinical data and IVF outcomes

At study entry, both male and female participants' date of birth was collected and their weight and height were measured by trained study staff. Body mass index (BMI) was calculated as weight (in kilograms) per height (in meters) squared. Clinical information on the IVF treatment cycle was collected or abstracted from the female partner's electronic medical record by trained study staff. Infertility diagnosis was physician determined according to the Society for Assisted Reproductive Technology (SART, 2013; Mok-Lin et al., 2010). IVF treatment protocols include: (1) luteal phase gonadotropin releasing hormone (GnRH) agonist (low-, regular-, or high-dose leuprolide acetate, Lupron), (2) follicular phase GnRH-agonist/Flare stimulation, or (3) GnRH-antagonist. Fertilization was confirmed 17–20 h after insemination by the presence of a fertilized oocyte with two pronuclei. For analysis we classified embryos as best quality if they had 4 cells on day 2, 8 cells on day 3, and a morphologic quality score of 1 or 2 on days 2 and 3 (Veeck and Zaninovic, 2003). Implantation was defined as a serum β-hCG level > 6 mIU/mL approximately 17 days (range 15–20 days) after egg retrieval, clinical pregnancy as the presence of an intrauterine pregnancy confirmed by ultrasound at approximately 6 weeks gestation, and live birth as the birth of a neonate on or after 24 weeks gestation.

2.3. PFR assessment in urine samples

Urine samples were provided during the IVF cycle, which for men was typically during the visit to provide a semen sample. All men included in this analysis provided a single urine sample per IVF cycle and women provided up to two urine samples per IVF cycle. Following collection of each sample, specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc.).

Extraction and analysis methods for BCIPP, BDCIPP, DPHP, ip-PPP and tb-PPP followed methods previously developed by Dr. Stapleton's laboratory at Duke University (Butt et al., 2014). Briefly, urine samples were thawed and a 2.5 to 5 ml aliquot was transferred to a clean glass test tube where it was spiked with mass-labeled internal standards (d_{10}-BDCIPP = 80 ng, d_{10}-DPHP = 60 ng). After acidifying to pH < 6.5 with formic acid, samples were diluted 1:1 with water and concentrated and cleaned using solid-phase extraction techniques (SPE). The SPE eluent was blown to dryness under a gentle nitrogen stream, reconstituted in 500 µl of 1:1 H_2O:MeOH and spiked with the recovery standard ($^{13}C_2$-DPHP = 81.5 ng). Extracts were analyzed by negative electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (Butt et al., 2014). Chromatography was achieved under gradient conditions using a Luna C18(2) column (50 × 2.0 mm, 2.5 µm particle size, Phenomenex, Torrance, CA) preceded by a SecurityGuard Polar-RP (4 × 2.0 mm) guard cartridge. The mobile phases were methanol and water (modified with 0.8 mM ammonium acetate), flow rate was 300 µl/min, the injection volume was 5 µl and the column oven was 45 °C. Data were acquired