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Endocrine disrupting chemicals in seminal plasma and couple fecundity

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ABSTRACT

Growing evidence supports the importance of men's exposure to non-persistent endocrine disruptors (EDCs) and couple fecundability, as measured by time-to-pregnancy (TTP). This evolving literature contrasts with the largely equivocal findings reported for women's exposures and fecundity. While most evidence relies upon urinary concentrations, quantification of EDCs in seminal plasma may be more informative about potential toxicity arising within the testes. We analyzed 5 chemical classes of non-persistent EDCs in seminal plasma for 339 male partners of couples who were recruited prior to conception and who were followed daily until pregnant or after one year of trying. Benzophenones, bisphenols, parabens, and phthalate metabolites and phthalate diesters were measured using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) except for phthalate diesters, which were analyzed using gas chromatography-mass spectrometry. Cox regression with discrete-time was used to estimate fecundability odds ratios (FORs) and 95% confidence intervals (CIs) for each chemical to estimate the probability of pregnancy. While most EDCs were detected in seminal plasma, concentrations were lower than urinary concentrations previously analyzed for the cohort. None of the EDCs were significantly associated with fecundability even after covariate adjustment, though benzophenones consistently yielded FORs < 1.0 (ranging from 0.72 to 0.91) in couple-adjusted models suggestive of diminished fecundity (longer TTP). The findings underscore that a range of EDCs can be quantified in seminal plasma, but the lower concentrations may require a large cohort for assessing couple fecundability, as well as the need to consider other fecundity outcomes such as semen quality.

1. Introduction

Endocrine disrupting chemicals (EDCs) are exogenous chemicals capable of interfering with any aspect of hormone action (Zoeller et al., 2012). Both persistent and non-persistent EDCs or those with long and short half-lives, respectively, have been associated with diminished fecundity, which is defined as the biologic capability of men and women for reproduction (Buck Louis, 2011a). To date, much of the existing research focusing on EDCs and fecundity has relied upon measured concentrations in women trying for pregnancy. Currently,

Abbreviations: BP-1, 2,4-dihydroxybenzophenone; BP-2, 2,2′,4,4′-tetrahydroxybenzophenone; BP-3, 2-hydroxy-4-methoxybenzophenone; BP-8, 2,2′-dihydroxy-4-methoxybenzophenone; 4-OH-BP, 4-hydroxybenzophenone; BPA, bisphenol A;; BPF, bisphenol F;; BPS, bisphenol S; BuP, butyl-paraben; BzBP, benzyl butyl phthalate; BzP, benzyl-paraben; CI, confidence interval; DBP, di-*n*-butyl phthalate; DCHP, dicyclohexyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DIBP, diisobutyl phthalate; DMP, dimethyl phthalate; DNHP, di-*n*-hexyl phthalate; DOP, di-*n*-octyl phthalate;; EDC, endocrine disrupting chemicals; EtP, ethyl-paraben; FOR, fecundability odds ratio; HeP, heptyl-paraben; 4-HB, 4-hydroxybenzoic acid; 3,4-DHB, 3,4-dihydroxybenzoic acid; MnBP, mono-n-butyl phthalate; MBzP, mono-benzyl phthalate; MCHP, mono-cyclohexyl phthalate; MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MIDP, mono-(8-methyl-1-nonyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MeP, methyl-paraben; MHxP, mono-hexyl phthalate; MIBP, mono-2-isobutyl phthalate; MINP, mono-isononyl phthalate; MMP, mono-methyl phthalate; MOP, mono-octyl phthalate; PrP, propyl-paraben; TCC, triclocarban; TCS, triclosan; TTP, time-to-pregnancy

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there is evidence suggesting diminished fecundability, as measured by a longer time-to-pregnancy, with increasing concentrations of various classes of persistent EDCs, including dioxins, organochlorine pesticides, perfluorochemicals, polybrominated diphenyl ethers, and polychlorinated biphenyls (Axmon et al., 2005; Eskenazi et al., 2010; Fei et al., 2009; Gesink Law et al., 2005; Harley et al., 2010). Less attention has focused on non-persistent EDCs and fecundability, and findings reported to date are largely equivocal. For example, specific parabens (Smarr et al., 2017a) and phthalates (Thomsen et al., 2017) have been reported to be associated with reduced fecundability or a longer TTP in prospective cohort studies with preconception enrollment of women or couples, whereas other such studies reported no associations (Buck Louis et al., 2014a; Jukic et al., 2016; Vélez et al., 2015). Also of note are reported associations between non-persistent EDCs and other fecundity endpoints such as alterations in hormonal profiles or menstrual cycles and poorer *in vitro* fertilization (IVF) outcomes, as recently reviewed (Mínguez-Alarcón and Gaskins, 2017).

Since human fecundity is a couple dependent outcome, it is important to assess EDC exposures in both partners of the couple. Recently, we summarized the findings from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study and noted that male partners' concentrations of both persistent and non-persistent EDCs were significantly associated with diminished couple fecundability or a longer TTP even in the absence of findings for female partners (Buck Louis et al., 2016). These collective findings underscore the importance of studying males when focusing on the relation between EDCs and couple fecundity. Findings from prospective IVF cohort studies also affirm the importance of studying the male partner for non-persistent EDCs such as phthalates, since negative associations have been observed between EDCs and implantation and live birth rates (Dodge et al., 2015).

Seminal fluid is a unique matrix for studying the reproductive effects of EDCs, since it is assumed to provide a more direct measure of within testes exposure (Vitku et al. (2015); whereas, urinary concentrations may be relatively more informative about total body burdens. Various classes of non-persistent EDCs have been detected in seminal plasma, such as benzophenones, bisphenol A, parabens, and phthalates (Bloom et al., 2015; Frederiksen et al., 2010, 2011; León et al., 2010; Vitku et al., 2016), and distributions have been compared across biologic media. For example, concentrations of 13 phthalate metabolites and 5 parabens were measured in the urine, serum and seminal plasma of 60 young Danish men. Urinary concentrations were higher than those in other matrices with relatively low correlations between urine and seminal plasma (Frederiksen et al., 2010, 2011). Another descriptive study found higher mean concentrations of 5 phthalates in the semen of 79 infertile men in comparison to 94 matched fertile men (Wang et al., 2015), and other authors have reported negative associations between seminal plasma concentrations of EDCs and semen quality (Chang et al., 2017; Vitku et al., 2016). These findings highlight the importance of assessing non-persistent EDCs in seminal plasma relative to human fecundity. Prompted by no previous research exploring this relation as known to us, we assessed a range of non-persistent EDCs measured in seminal plasma and couple fecundability, as measured by TTP. We compare the results to earlier findings for this cohort based upon urinary concentrations to assess the consistency of findings by biologic media.

2. Materials and methods

2.1. Design and study population

A prospective cohort design with preconception recruitment of couples (n = 501) was used to recruit 501 eligible couples who were discontinuing contraception to try for pregnancy from 16 counties in Michigan and Texas between 2005 and 2009. Given the absence of established sampling frameworks for identifying couples planning pregnancies, we used fishing/hunting license registries and marketing databases for these interests to develop samples. Households were called and residents were screened for eligibility. By design, the eligibility criteria for the male partner were minimal: aged 18 + years of age, able to communicate in English or Spanish and no history of clinically diagnosed infertility. Couples were followed daily until pregnancy or 12 months of trying without pregnancy. The study cohort for this analysis was restricted to male partners of couples with an observed TTP while participating in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, and who had residual semen samples of sufficient volume for quantifying non-persistent EDCs in seminal plasma (n = 339; 68%). A complete description of the LIFE Study's design and methods is provided elsewhere (Buck Louis et al., 2011b).

2.1.1. Data and biospecimen collection

Male partners were interviewed upon enrollment into the cohort to capture lifestyle and medical and reproductive history, and were subsequently trained in the completion of daily journals focusing on lifestyle while the couple was trying for pregnancy. Trained research assistants weighed men and measured their height using standardized methods and calibrated scales and measuring tapes for the calculation of body mass index (BMI; weight in kg / height in m^2). After the baseline interview, all men provided blood and urine samples for the quantification of persistent and non-persistent EDCs, respectively. In addition, men were instructed in the collection of two at home semen samples with the intent of assessing semen quality. The first sample was obtained the day following the interview and the second sample approximately 1 month later. The second sample was used for an abbreviated semen analysis in part to corroborate azoospermia found in the first and more in-depth semen analysis, and for the quantification of EDCs. Men were instructed to collect the sample without the use of lubricants following 2 days of abstinence, and to return the sample to the andrology laboratory using overnight delivery, as previously described (Buck Louis et al., 2014b). Residual samples were stored as a pellet and then thawed and separated into sperm and seminal plasma for the quantification of non-persistent EDCs in seminal plasma by laboratory personnel experienced in the processing of semen. Specifically, seminal plasma was separated from sperm by centrifuging samples at 3000 rpm for 10 min. Seminal plasma was then pipetted for analysis. The interval between urine and semen collection was on average 2 months. Full human subjects' approval was obtained from all participating institutions, and men gave informed consents prior to any data or biospecimen collection.

2.1.2. Toxicological analysis

The following EDCs were quantified in approximately 1.5 mL seminal plasma: 3 bisphenols [bisphenol A (BPA), bisphenol F (BPF) and bisphenol S (BPS)]; 5 benzophenones [2,4-dihydroxybenzophenone (BP-1), 2,2',4,4'-tetrahydroxybenzophenone2,2′,4,4′-tetrahydroxybenzophenone (BP-2), 2-hydroxy-4-methoxybenzophenone (BP-3), 2,2'-dihydroxy-4-methoxybenzophenone2,2′-dihydroxy-4-methoxybenzophenone (BP-8), and 4-hydroxybenzophenone (4-OH-BP)]; 9 environmental phenols [triclosan (TCS), methyl-paraben (MeP), ethyl-paraben (EtP), propyl-paraben (PrP), butyl-paraben (BuP), heptyl-paraben (HeP), benzyl-paraben (BzP), and metabolites 4-hydroxybenzoic acid (4-HB) and 3,4-dihydroxybenzoic acid (3,4-DHB)]; 15 phthalate metabolites [mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-[(2-carboxymethyl) hexyl] phthalate (MCMHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP),mono-(3-carboxypropyl) phthalate (MCPP), monomethyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-2-isobutyl phthalate (MIBP), mono-hexyl phthalate (MHxP), mono-cyclohexyl phthalate (MCHP), mono-octyl phthalate (MOP), mono-isononyl phthalate (MINP), mono-benzyl phthalate (MBzP),and mono-(8-methyl-1-nonyl) phthalate (MIDP)]; and 9 phthalate diesters [dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl