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The association between urinary concentrations of phosphorous-containing flame retardant metabolites and semen parameters among men from a fertility clinic

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Abstract

Background—The use of PFRs has steadily increased as brominated compounds have been or are being phased out. Human exposure is widespread and animal studies have shown adverse impacts on male reproduction, but human data are lacking.

Objective—To study the associations between urinary concentrations of phosphorous-containing flame retardant (PFR) metabolites and semen parameters.

Methods—A subset of 220 men from an existing longitudinal cohort of couples were recruited from Massachusetts General Hospital fertility clinic between 2005 and 2015. Semen parameters

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included sperm count, concentration, motility, and morphology; some men had samples measured from multiple clinic visits (up to five visits; n=269 semen samples). 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)] were measured in urine samples (between one and five urine samples per participant; n=355 urine samples). Semen parameters were evaluated continuously and dichotomized for models. Metabolites were assessed for associations with semen parameters as continuous and categorized into quartiles using multivariable generalized mixed models, adjusted for specific gravity, age, BMI, smoking, and abstinence period.

Results—Metabolites BDCIPP, DPHP, and ip-PPP were detected in a high proportion of urine samples (85%, 86%, and 65% respectively). Concentrations varied by season of collection, particularly for BDCIPP where samples collected in the summer were approximately 2-fold higher than concentrations of other seasons ($p < 0.0001$). The odds of having a sperm count less than 39 mil/ejaculate decreased by 20% for increasing BDCIPP concentrations ($p = 0.04$). When regressing semen parameters on PFR metabolite quartiles, some negative associations were observed for individual quartiles among sample volume and morphology, but overall associations were weak and inconsistent.

Conclusion—Detection rates were high for BDCIPP, DPHP, and ip-PPP. We did not observe consistent associations between PFR metabolites and semen parameters. Due to the high prevalence of exposure, further investigation of other potential health effects should be conducted.

Keywords

Phosphorous-containing flame retardants; semen quality; male fertility

Introduction

Infertility, the inability to conceive after one year of unprotected intercourse, affects approximately one out of every six couples (Meacham *et al.*, 2007). In 2002, a national survey estimated two million couples in the U.S. suffer from infertility (Chandra A *et al.*, 2002). An increase in infertility is partially related to the postponement of first birth (Dunson *et al.*, 2004; Sharma *et al.*, 2013). However, aside from advanced age, genetic risk factors, psychosocial factors, and environmental agents can also impair fertility (Chalupka and Chalupka, 2010; Macaluso *et al.*, 2010).

The underlying cause of infertility may be related to female or male factors or a combination of both. In 2002, approximately 20% of men reported fertility problems (Hotaling *et al.*, 2012). However, a national survey study suggests this to be an underestimate for the U.S. population as male factor infertility is likely to be underdiagnosed (Hwang *et al.*, 2011; Hotaling *et al.*, 2012) Although, a recent meta-analysis found an approximate 50% reduction in total sperm count and sperm concentration among men from Western countries over the last several decades, irrespective of fertility diagnosis (Levine *et al.*, 2017). The cost of male factor infertility alone was \$17 billion US dollars in the year 2000, which does not include the additional \$18 billion for assisted reproductive technology treatment (Meacham *et al.*, 2007). To date, a semen analysis measuring sperm count, concentration, morphology, and

volume remains the primary evaluation for male factor infertility (World Health Organization (WHO), 2010; Hwang *et al.*, 2011). Semen quality is also associated with other various health outcomes. A study of Finnish men found an increase risk in testicular cancer among those with poor semen quality (Jørgensen *et al.*, 2011), while a Danish study found subpar semen associated with a shorter life span (Jensen *et al.*, 2009). Many environmental agents such as glycol ethers, pesticides, and phthalates are also known to impact semen quality (Chalupka and Chalupka, 2010).

Among possible environmental chemicals of concern for reproductive health are organophosphate esters, which are increasingly being used as flame retardants (PFRs). The use of PFRs has grown due to their use as replacement chemicals for the phased-out of polybrominated diphenyl ethers. As their prevalence rose, PFRs became and remain a high production volume chemical. Today they are commonly applied to materials for use as either a flame retardant, or as a plasticizer, therefore are common in polyvinyl chloride (PVC), hydraulic fluids, and polyurethane foam (PUF) in cars and furniture (Marklund *et al.*, 2003; van der Veen and de Boer, 2012; Tajima *et al.*, 2014). PFRs include both chlorinated alkyl esters such as tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP), and non-halogenated aryl phosphates such as triphenyl phosphate (TPHP) (Marklund *et al.*, 2003; Brommer and Harrad, 2015). Often considered ‘additive’ compounds, the weak bonds allow volatilization into air and settlement in dust. PFRs have been detected in the dust of homes, cars, and offices (Brommer and Harrad, 2015; Ali *et al.*, 2016). Unlike brominated flame retardants, PFRs are considered non-persistent, with a short half-life in humans, yet they are detected in nearly 100% of urine samples from men (Meeker *et al.*, 2013a), pregnant women (Hoffman *et al.*, 2014), and children (Cequier *et al.*, 2015).

To date studies assessing the health effects of PFRs are limited, yet animal and in vitro studies suggest these compounds act as endocrine disrupting chemicals. A study of TPHP and tris(2-chloroethyl) phosphate (TCEP) in mice found a disruption of gene expression for testosterone synthesis and oxidative stress (Chen *et al.*, 2015), while an in vitro study of mouse Leydig cells found a disruption in steroid production (Schang *et al.*, 2016). A small study of U.S. men detected inverse relationships of bis(1,3-dichloropropyl) phosphate (BDCPP) and diphenyl phosphate (DPHP) concentrations in urine with sperm concentration and motility (Meeker *et al.*, 2013b). To the best of our knowledge, this prior analysis is the only human study to date to assess the relationship of PFRs with semen parameters. In our present work, we expand upon this preliminary evidence with a larger cohort to characterize the relationship between five PFR metabolites: bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), isopropylphenyl phenyl phosphate (ip-PPP), tert-butylphenyl phenyl phosphate (tb-PPP) with semen parameters in men attending a fertility center.

Methods

Participant Recruitment

Participants from this analysis are a subset of men from the Environment and Reproductive Health (EARTH) study, a larger cohort assessing the impact of environmental agents on

reproductive health. Participation and recruitment have been described elsewhere (Meeker *et al.*, 2006). Briefly, men (18–54 years of age) attending the Massachusetts General Hospital fertility clinic between 2005 and 2015 were eligible. Participants originated from couples whose infertility diagnosis was either male factor, female factor, or a combination of both. Prior vasectomy or hormone supplementation were the only exclusion criteria. Informed consent was signed by each participant and Institutional Review Board approval was received by all institutions.

Semen Collection and Analysis

Semen collection and analysis have been previously described (Meeker *et al.*, 2006; Lewis *et al.*, 2017). Briefly, men abstained from ejaculation for 48 hours prior to sample collection into plastic specimen cup. Men provided up to five samples depending on the number of fertility treatments, additional fertility evaluation, or a combination of both. An andrologist quantified sample volume (mL) with a graduated pipet. Sperm concentration (mil/mL) and motility (% motile) was determined using a computer-aided semen analyzer (CASA, version 10 HTM-IVOS; Hamilton Thorne Research, Beverly, MA). Samples (5 μ L) were collected on a disposable Leja Slide (Spectrum Technologies, CA, USA) and placed into a pre-warmed (37°C) counting chamber (Sefi-Medical Instruments, Haifa, Israel) before assessing concentration and motility. Among each sample, at least 200 sperm cells were analyzed from four different fields. Progressive motility was graded in accordance to the WHO's assessment criteria of active movement (linearly or in a large circle), regardless of velocity (WHO, 2010). The product of sperm concentration and sample volume determined sperm count (mil/ejaculate) while progressive motility count (mil/ejaculate) was calculated by multiplying progressive motility and total sperm count. Fresh semen samples were allowed to dry on two prepared slides and prepared for morphology (% normal) assessment with a microscope using an oil-immersion 100 \times objective (Nikon, Tokyo, Japan). A minimum of 200 cells per slide were analyzed for each specimen. Classification of normal or subnormal morphology was determined using strict Kruger scoring criteria (Kruger *et al.*, 1988). Quality assurance and control procedures in the laboratory were conducted for sperm morphology smears weekly, as well as quarterly and biannual evaluations for technicians.

Urine Collection and Analysis

Urine samples (up to five cycles) were collected in sterile polypropylene cups on the day of oocyte retrieval for each cycle per participant. Prior to being frozen (-80°) and stored, specific gravity (SG) was measured using a handheld refractometer (National Instrument Company, Inc., Austin, TX). For metabolite analysis, samples were shipped overnight on dry ice to Dr. Stapleton's lab at Duke University (Durham, NC).

Analytic methods for metabolites: BCIPP, BDCIPP, DPHP, ip-PPP, and tb-PPP have been previously described (Butt *et al.*, 2014). Briefly, 5 ml aliquots were thawed and transferred to test tubes and spiked with internal standards (d_{10} -BDCIPP = 80 ng, d_{10} -DPHP = 60 ng) before being acidified (pH <6.5) with formic acid and diluted with 1:1 with water. Solid phase extraction (SPE) was used to concentrate and clean samples before drying via nitrogen stream and spiked with the recovery standard ($^{13}C_2$ -DPHP = 81.5 ng). Extracts were analyzed using negative electrospray ionization liquid chromatography tandem mass

spectrometry (LC-MS/MS) detailed previously (Butt *et al.*, 2014). Optimal parameters under multiple reaction conditions were used to acquire data. The internal standard used for BCIPP and BDCIPP was d₁₀-BDCIPP, while quantification of DPHP, ip-DPHP, and tb-DPHP was performed using d₁₀-DPHP.

Quality assurance and control procedures for LC-MS/MS have been described previously (Carignan *et al.*, 2017). Briefly, samples were processed in multiple batches including five blanks per batch (5 ml Milli-Q water); each batch providing a distinct method detection limit (MDL). MDLs were designated as three times the standard deviation of laboratory blanks and ranged from: 0.07–0.17 pg/ml for BCIPP, 0.02–0.11 pg/ml for BDCIPP, 0.09–0.18 pg/ml for DPHP, 0.06–0.12 pg/ml for ip-PPP, and 0.04–0.15 pg/ml for tb-PPP. Urine samples from previous studies were pooled to establish a standard reference material (SRM) and routinely analyzed. Duplicates of two-subsamples were analyzed to evaluate precision.

Statistical Analysis

Descriptive statistics for PFR metabolites, semen parameters, and demographic factors were calculated. Values below MDL for metabolites were imputed as MDL/ 2. Metabolites were presented as wet-weight and adjusted for SG as: $C_{SG} = C * [(SG_M - 1) / (SG_i - 1)]$, where C_{SG} = SG-adjusted urinary metabolite concentration, C = urinary metabolite concentration, SG_M = mean SG for the population, and SG_i = SG for an individual sample (Boeniger *et al.*, 1993). We evaluated bivariate associations among PFR metabolites, semen parameters, and demographic factors using Spearman correlation coefficients, Wilcoxon rank-sum tests, and Kruskal-Wallis tests as appropriate. A sum variable (Σ PFR) for BDCIPP, DPHP, and ip-PPP was created by combining all three metabolites per sample. Intraclass correlation coefficients (ICC) and 95% confidence intervals for metabolites (wet-weight and SG corrected) and semen parameters were calculated to assess variability between samples of each participant. All metabolites and semen parameters presenting as right-skewed were transformed by natural logarithm for further statistical modeling. PFR metabolites were evaluated as continuous variables and quartiles except for tb-PPP with low detection rate (11.34 %) was modeled as detect/non-detect. Sperm parameters were evaluated both continuously and dichotomized using WHO reference level for sperm: count (< 39mil/ ejaculate), concentration (< 15 mil/mL), motility (< 40%), progressive motility (<32%), and morphology (< 4% normal) (WHO, 2010). Initially, crude associations were calculated among PFR metabolites and semen parameters (Supplemental Table 1). Bivariate tests for possible covariates: age, BMI, abstinence period, race, smoking status, education, and season of collection along with priori knowledge were used to select covariates for modeling (Supplemental Table 2). Although season of sample collection was associated with PFR metabolites, it was not associated with semen parameters and not included in final models. Multivariable regression models, adjusted for SG, age, BMI, and abstinence period, were used to test associations using only the first urine and semen sample for all participants. Multivariable generalized mixed models using continuous, dichotomous, and quartiles for PFRs were used to assess associations with repeated exposures and/or semen parameters. To test for trends, quartiles of each metabolite were treated as a continuous variable. We conducted a sensitivity analysis excluding SG measurements below 1.01 and above 1.03 for multivariable models (Supplemental Table 3) to examine any effect of extreme urine

concentrations. Missing data were excluded from models. All statistical analyses were carried out using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Study Population

Demographic characteristics of our subsample from the EARTH study are displayed in Table 1. Demographics from this sample are similar to previous studies in similar cohorts (Meeker *et al.*, 2006) as well as national trends of men undergoing IVF (Hotaling *et al.*, 2012) regarding age (mean=36.66 ± 5.07), BMI (27.18 ± 4.03), race or ethnicity (89% white), and education (80% college graduates).

The distribution of semen parameters among our sample is presented in Table 2. All parameters met WHO guidelines for normal sperm among more than half of participants (WHO, 2010). Total sperm count and concentration exceeded the guideline for normal sperm (43.42 and 15.9, respectively) in 90% of samples. Conversely, less than half of participants had above average motility (45%) while 75% exceeded the guideline for morphology (4%). Participants provided semen (n=269) and urine (n=355) samples from up to five clinic visits, with the majority of men providing one to three. Repeated measures of semen parameters had moderately strong intraclass correlations (0.51–0.58), except motility (ICC=0.79) and progressive motility (ICC=0.71) which had stronger ICCs (Supplemental Table 4).

Distributions of PFR metabolites, as both wet-weight and SG-corrected are displayed in Table 3. Metabolites BDCIPP, DPHP, and ip-PPP were detected in a high proportion of urine samples (85%, 86%, and 66% respectively). We identified weak ($r < 0.30$) yet significant ($p < 0.01$) correlations among BDCIPP, DPHP, and ip-PPP, and moderate ($r=0.43$) correlations between DPHP and tb-DPHP ($p=0.01$) (Data not shown). Similarly, temporal stability between metabolite measurements (Table 4) were weak-to-moderate (ICC < 0.35) and decreased further when excluding non-detects and adjusting for SG. Concentrations varied by season of collection, particularly for BDCIPP where samples collected in the summer had the highest concentrations ($p < 0.0001$) (Supplemental Table 2).

When modeling PFR metabolites and semen parameters as continuous variables, there were no significant effect estimates from repeated measure models (Table 5). Whereas when semen parameters were dichotomized, elevated BDCIPP was associated with a decreased odds of low sperm count (OR=0.79, 95% CI=0.64, 0.99; $p=0.04$). Results were similar in a sensitivity analysis excluding extreme urine dilution concentrations (0.01 SG 1.03) (Supplemental Table 3). When modeling PFR metabolites as quartiles, we identified several negative associations among individual quartiles. DPHP (Quartile 2, $p=0.04$) and ΣPFR (Quartile 3, $p=0.03$) (Table 6) concentrations were inversely associated with sample volume, while concentrations of DPHP (Quartile 3, $p=0.02$) increased the odds abnormal semen morphology (Supplemental Table 5). However, overall p-values were not statistically significant. When the semen parameters were modeled continuously (Table 6), concentrations in the third quartile for metabolites BDCIPP, ip-PPP, and Σ PFR had the

strongest decrease in sample volume, yet when they were dichotomized (Supplemental Table 5), results were mixed across all parameters.

Discussion

Although exposure was prevalent, overall we did not observe consistent associations between PFR metabolites and semen parameters. To our knowledge, this is the largest study to evaluate the relationship between phosphorous-containing flame retardant metabolites and semen quality. Most semen parameters in our sample were above established reference levels (WHO, 2010) and within-participant reliability was moderate-to-strong for repeated samples. Metabolites BDCIPP, DPHP, and ip-PPP were detected at high rates in urine and temporal reliability of repeated samples within participant was weak-to-moderate. While we found a decreased odds of a low sperm count (<39 mil/ejaculate) with increasing BDCIPP concentrations, overall associations were weak and inconsistent.

Comparisons with other studies

To date, there are limited studies examining the potential for adverse health effects related to PFR exposure despite their high detection in various environmental media and respective metabolites in urine. Parent compounds TDCPP and TPHP were detected in nearly all samples of house dust from a previous sample of 50 men from the EARTH cohort (Meeker and Stapleton, 2009). Similarly, a study in Durham, North Carolina (n=40 adults) detected parent compounds to BDCIPP, BCIPP, and DPHP (TDCIPP, TCIPP, and TPHP, respectively) in 100% of samples using silicone wrist bands and >95% of hand wipes (Hammel *et al.*, 2016). Concentrations are showing temporal progression; a recent study combining several cohorts from various parts of the U.S. found a 15-fold increase in BDCIPP samples collected in 2015 compared to those collected in 2002 (Hoffman *et al.*, 2017a).

Studies characterizing PFRs in male populations are insufficient compared to those among women and children. Yet, analogous to high detection rate in environmental media, metabolites BDICPP and DPHP were detected in >90% of individuals (Meeker *et al.*, 2013a) and >95% of pooled samples (Van den Eede *et al.*, 2015). Concentrations of BDCIPP in our samples were six-fold higher compared to a prior study (n=16) of adults in California (Median=0.09 ng/mL) (Dodson *et al.*, 2014). Our samples of DPHP were also twice as high (Median= 0.44 ng/mL), yet both were similar in having low detection of BCIPP. A small sample (n=29) of office workers in Boston, MA also had slightly lower concentration of BDCIPP (SG-adjusted Mean=408 pg/mil) (Carignan *et al.*, 2013). However, distributions of BDCIPP, DPHP, and ip-PPP were similar to a recent study of 211 females (n=563 samples) from the EARTH cohort (SG-adjusted Mean= 0.66, 0.78, 0.22 µg/L respectively) (Carignan *et al.*, 2017). Weak to moderate stability in repeated measurements in our sample were somewhat lower than reported from a previous study for BDICPP (ICC=0.55–0.72) and DPHP (ICC=0.35–0.51), although the sample period was considerably shorter (3 months) (Meeker *et al.*, 2013a). We found an unexpected relationship with PFR concentrations and season of sample, where concentrations of BDCIPP (p<0.0001) were highest in summer (June–August), while DPHP (p=0.05) concentrations were highest in the winter (December–

February). A sample of adults spanning the US observed a similar seasonal relationship as BDCIPP concentrations in summer were 4.13 times higher than winter samples and contrary to our observations, DPHP concentrations were also highest in summer (Hoffman *et al.*, 2017a). Similar results were found among a sample of pregnant women, where summer (June–August) concentrations of BDCIPP and DPHP were almost 4-fold and 60% higher, respectively compared to winter samples (Hoffman *et al.*, 2017b).

Limited research has been conducted on PFR metabolites and male reproductive health. However, we previously reported a decrease in sperm morphology (36%), straight-line velocity (18%), and curvilinear velocity (14%) in association with BDCIPP in a previous study (n=33) men from the EARTH cohort. The same study also reported decreased sperm concentration (57%) and straight-line velocity (19%) in association with urinary DPHP (Meeker *et al.*, 2013b). Similar relationships were detected in a study (n=50) of their parent compounds in house dust where concentrations of TDCPP and TPHP were inversely associated with sperm concentration, motility, and morphology, although only the relationship between TPHP and sperm concentration was statistically significant (p=0.01) (Meeker and Stapleton, 2009). In this more robust analysis we observed suggestive declining trends in our adjusted models among BDCIPP and DPHP with total sperm count and sample volume when modeled as continuous variables. Our observations are inconsistent with previous work, possibly as a result of substantial sample size differences.

Animal and in-vitro studies

Laboratory studies assessing the reproductive impacts of PFRs are also limited, yet suggest PFRs act as endocrine disruptors and induce oxidative stress. Several in vitro models found TDCPP to be an estrogen agonist (Kojima *et al.*, 2013; Krivoshiev *et al.*, 2016) while another found the hydroxylated metabolite of TPHP to have stronger estrogenic activity than the parent compound (Kojima *et al.*, 2016). A study of mouse Leydig cells concluded TPHP failed to disrupt steroidogenesis, although increased TPHP concentrations resulted in a 1.7 fold increase in superoxide production (Schang *et al.*, 2016). However, another study of mice found TCPP and TECP to alter antioxidant enzymes and testosterone levels (Chen *et al.*, 2015).

Limitations

Although novel, our study is not without limitations. While the largest study to date, our sample size is somewhat modest. Men from a fertility clinic are a selective population that potentially limit their generalizability to the men from general population (Hotaling *et al.*, 2012). However, the semen quality of these men is comparable with the semen quality of men from the general population. Due to limited studies characterizing PFRs in male cohorts, we are unable to conclude the PFR concentrations found in our study do not reflect levels in the general population or that men from a fertility clinic would respond differently to PFR exposure. Concentrations of PFRs in our sample are similar to those measured among the female partners of the EARTH study cohort (Carignan *et al.*, 2017), yet considerably lower compared to more recent samples of pregnant women in Durham, North Carolina (Hoffman *et al.*, 2017b) and Shanghai, Chania (Feng *et al.*, 2016). Contrary to PBDEs which have a long half-life, PFRs are less-persistent and samples are subject to

exposure misclassification since urinary metabolite levels may reflect exposure only hours or days prior to sample collection. However, we attempted to reduce this source of error by collecting up to five urine samples per participant and previously reported levels remain moderately stable over a three month period (Meeker *et al.*, 2013a). Finally, while we analyzed five commonly used PFR metabolites, there are other PFRs in use that should be the focus of future investigations. Thus, our results cannot conclusively determine a lack of association with all PFR metabolites.

Conclusion

The results of the relationship between PFRs and male reproductive health from our study are inconclusive. Although our findings were inconsistent, we observed high detection of metabolites which coincides with previous and concurrent studies. In comparison to our results and other studies, concentrations of PFR metabolites appear to be increasing over time. Widespread detection rates, temporal increases in concentrations, along with evidence from animal research establish the necessity for additional investigation of PFRs on male reproductive health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Demographic characteristics among 220 men from the Environment and Reproductive Health (EARTH) cohort

Table 1

Characteristic	N	Mean or %	SD	Median	25 th , 75 th quartiles
Age	218	36.66	5.07	35.97	32.92, 39.86
BMI	217	27.18	4.03	26.85	24.30, 29.07
Abstinence period	186	3.91	13.87	2.42	1.83, 3.04
Race					
White	194	88.99			
Black	4	1.83			
Asian	13	5.96			
Other	7	3.21			
Smoking Status					
Never smoke	153	70.18			
Past smoker	52	23.85			
Current smoker	13	5.96			
Education					
<High school	3	1.65			
HS grad	6	3.30			
1 or 2 yr. college	12	6.59			
3 or 4 yr. college	11	6.04			
College grad.	61	33.52			
Graduate degree	89	48.90			
Season of sample					
Winter	79	23.58			
Spring	79	23.58			
Summer	90	26.87			
Fall	87	25.97			

SD: standard deviation; Missing: age, race, smoking n=2; BMI n=3; Abstinence period n=34; Education n=38; Season: n=355 using all observations; Winter: December–February, Spring: March–May; Summer: June–August; Fall: September–November

Table 2

Distribution of semen parameters among 220 men

Semen Parameter	N	Mean	Percentiles							
			10 th	25 th	50 th	75 th	90 th	95 th	Max	
Total sperm count(mil/ejaculate)	235	172.48	43.42	68.04	133.25	224.80	364.48	511.27	679.14	
Concentration (mil/mL)	237	77.02	15.9	30.2	58.2	110.1	140.5	156.5	617.4	
Motility (P+NP) (%)	237	44.30	10	25	45	63	75	80	93	
Progressive motility (%)	234	24.89	5	12	24	36	45	49	69	
Morphology (% normal sperm)	255	6.18	2	4	6	8	10	12	18	
Sample volume (mL)	267	2.68	1.0	1.7	2.5	3.5	4.5	5.1	8.7	

P+NP: Progressive + Non progressive semen motility

Table 3 Distribution of uncorrected and Specific gravity-corrected PFR metabolites (µg/L) of 220 men (n=355 urine measurements)

Wet-weight	N ≥MDL, (%)	GM	(95% CI)	Percentiles					
				25 th	50 th	75 th	90 th	95 th	Max
BDCIPP	285 (85.07)	0.62	(0.55, 0.71)	0.33	0.60	1.38	2.72	3.57	10.30
DPHP	289 (86.27)	0.78	(0.71, 0.87)	0.42	0.74	1.31	2.47	4.13	10.57
ip-PPP	223 (66.57)	0.35	(0.32, 0.39)	<MDL	<MDL	0.62	0.93	1.42	4.56
tb-DPHP	38 (11.34)	0.16	(0.12, 0.20)	<MDL	<MDL	<MDL	0.38	0.73	2.24
SG Adjusted									
BDCIPP	285 (85.07)	0.64	(0.57, 0.73)	0.35	0.61	1.14	2.37	4.26	20.24
DPHP	289 (86.27)	0.77	(0.70, 0.84)	0.46	0.70	1.15	2.38	3.59	15.55
ip-PPP	223 (66.57)	0.32	(0.29, 0.35)	<MDL	<MDL	0.51	0.84	1.14	4.08
tb-DPHP	38 (11.34)	0.17	(0.12, 0.23)	<MDL	<MDL	<MDL	0.50	1.58	1.87
Specific gravity	-	0.017	(0.016, 0.018)	1.011	1.018	1.024	1.027	1.028	1.038

MDL: Method detection limit; GM: Geometric mean; BCIPP data not shown (n=3 measurements)

Intraclass correlation coefficients (95% CI) for uncorrected and SG corrected repeated urinary PFR metabolites

Table 4

Metabolite	All samples ^a		Excluding non-detects	
	Uncorrected	SG Adjusted	Uncorrected	SG Adjusted
BDCIPP	0.34 (0.20, 0.51)	0.21 (0.08, 0.45)	0.30 ^b (0.16, 0.50)	0.18 ^b (0.05, 0.47)
DPHP	0.07 (0.00, 0.68)	0.09 (0.01, 0.62)	0.07 (0.00, 0.68)	0.06 ^c (0.00, 0.81)
ip-PPP	0.37 (0.24, 0.52)	0.25 (0.11, 0.49)	0.28 ^d (0.13, 0.48)	0.13 ^d (0.02, 0.55)
ΣPFR	0.24 (0.11, 0.44)	0.19 (0.05, 0.49)	0.20 ^e (0.08, 0.43)	0.017 ^e (0.04, 0.50)

^a n=335 samples from 220 men;

^b n=285 samples from 187 men;

^c n=289 samples from 200 men;

^d n=233 samples from 167 men;

^e n=321 samples from 213 men

Table 5 Regression coefficients and odds ratios (95% CI) for semen parameters of men contributing (1–5) urine samples. Adjusted for specific gravity, age, BMI, smoking status & abstinence period

Sperm Parameter	PFR Metabolites ^a											
	BDCIPP			DPHP			ip-PPP			Σ PFR		
	β	95%CI	p-Value	β	95%CI	p-Value	β	95%CI	p-Value	β	95%CI	p-Value
Total sperm count (milL) ^a	-0.02	(-0.10, 0.07)	0.70	-0.01	(-0.11, 0.09)	0.82	-0.04	(-0.14, 0.07)	0.52	5.0×10 ⁻⁴	(-0.12, 0.13)	0.99
Concentration (mil/mL) ^a	0.002	(-0.08, 0.08)	0.96	0.005	(-0.09, 0.10)	0.92	-0.004	(-0.10, 0.09)	0.94	0.02	(-0.09, 0.14)	0.66
Motility (P+NP) (%) ^a	0.005	(-0.07, 0.08)	0.90	0.04	(0.05, 0.13)	0.40	0.12	(-0.08, 0.11)	0.72	0.03	(-0.08, 0.14)	0.55
Progressive motility ^a	0.04	(-0.04, 0.12)	0.30	0.03	(-0.06, 0.12)	0.53	0.05	(-0.04, 0.14)	0.28	0.06	(-0.05, 0.17)	0.25
Morphology (% norm)	0.17	(-0.16, 0.50)	0.30	0.12	(-0.31, 0.55)	0.58	0.21	(-0.21, 0.64)	0.32	0.20	(-0.29, 0.69)	0.42
Sample volume (mL)	-0.05	(-0.18, 0.08)	0.45	-0.08	(-0.24, 0.08)	0.31	-0.04	(-0.20, 0.12)	0.61	-0.12	(-0.31, 0.07)	0.20
Odds Ratio	OR			OR			OR			OR		
Total sperm count <39 mil/ejaculate	0.79	(0.64, 0.99)	0.04	0.93	(0.55, 1.55)	0.78	1.04	(0.63, 1.72)	0.88	0.75	(0.48, 1.16)	0.19
Sperm concentration <15 mil/mL	0.90	(0.72, 1.13)	0.37	0.97	(0.60, 1.57)	0.90	0.92	(0.54, 1.57)	0.76	0.82	(0.51, 1.32)	0.41
Percent motile sperm (P+NP) <32	1.07	(0.86, 1.35)	0.53	1.13	(0.84, 1.51)	0.42	1.04	(0.79, 1.38)	0.78	1.08	(0.78, 1.51)	0.64
Percent motile sperm (P+NP) <40	1.05	(0.84, 1.31)	0.63	1.01	(0.76, 1.35)	0.92	0.90	(0.66, 1.21)	0.48	0.92	(0.66, 1.28)	0.63
Percent morph. Sperm <4	0.92	(0.70, 1.20)	0.53	1.14	(0.83, 1.57)	0.44	0.94	(0.68, 1.30)	0.71	0.94	(0.64, 1.40)	0.78

^a natural log transformation

Table 6

Regression coefficients (95% CIs) by quartile of PFR metabolite for males contributing 1–5 samples. Adjusted for specific gravity, age, BMI, smoking status & abstinence period

PFR ^a	Semen parameters						
	Total sperm count ^a	Concentration (mil/mL) ^a	Motility (P+NP) (%) ^a	Progressive motility ^a	Morphology (% norm)	Sample volume (mL)	
BDCPP							
Q1	-	-	-	-	-	-	-
Q2	0.12	(-0.17, 0.35)	-0.01	-0.02	(-0.27, 0.24)	0.97	(-0.15, 2.10) 0.23
Q3	-0.04	(-0.34, 0.27)	0.09	0.01	(-0.26, 0.28)	0.39	(-0.81, 1.59) -0.25
Q4	0.04	(-0.29, 0.37)	0.03	0.19	(-0.11, 0.49)	1.07	(-0.15, 2.29) -0.05
p-trend	0.93	0.88	0.61	0.17		0.18	0.10
DPHP							
Q1	-	-	-	-	-	-	-
Q2	-0.01	(-0.29, 0.26)	0.23	0.16	(-0.09, 0.40)	0.32	(-0.82, 1.45) -0.45
Q3	-0.16	(-0.47, 0.14)	0.01	0.09	(-0.18, 0.35)	0.08	(-1.17, 1.33) -0.22
Q4	-0.02	(-0.36, 0.31)	0.12	0.08	(-0.22, 0.37)	0.09	(-1.23, 1.41) -0.40
p-trend	0.09	0.11	0.11	0.11		0.42	0.07
ip-PPP							
Q1	-	-	-	-	-	-	-
Q2	0.04	(-0.23, 0.32)	0.05	-0.07	(-0.31, 0.16)	-0.69	(-1.81, 0.43) -0.14
Q3	0.04	(-0.26, 0.34)	0.17	0.06	(-0.20, 0.32)	0.19	(-1.02, 1.40) -0.28
Q4	-0.15	(-0.49, 0.19)	-0.05	0.11	(-0.19, 0.41)	0.50	(-0.82, 1.81) -0.21
p-trend	0.07	0.10	0.10	0.14		0.67	0.09
ΣPFR							
Q1	-	-	-	-	-	-	-
Q2	-0.15	(-0.44, 0.14)	0.03	0.0005	(-0.24, 0.25)	0.33	(-0.81, 1.47) -0.19
Q3	-0.05	(-0.38, 0.28)	0.23	0.20	(-0.09, 0.48)	0.02	(-1.26, 1.31) -0.54
Q4	0.01	(-0.32, 0.34)	0.08	0.25	(-0.03, 0.54)	1.02	(-0.27, 2.30) -0.19
p-trend	0.12	0.12	0.16	0.18		0.73	0.10

^a natural log transformation; Quartile 1=reference