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ORIGINAL ARTICLE ***Reproductive epidemiology***

Association between use of phthalate-containing medication and semen quality among men in couples referred for assisted reproduction

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STUDY QUESTION: Does phthalate exposure from prescription drugs affect semen quality?

SUMMARY ANSWER: Exposure to phthalate-containing drugs is associated with poor semen quality.

WHAT IS KNOWN ALREADY: Phthalates and their metabolites have been shown to disrupt the hormone signalling in animal studies. One study has shown associations between medicinal phthalate exposure and poor semen quality, suggesting similar effects in humans.

STUDY DESIGN, SIZE, DURATION: We included 18 515 males with poor semen quality (cases) and 31 063 males with normal semen quality (controls) registered in the Danish IVF Registry from 2006 to 2016.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Exposure to phthalate-containing drugs was assessed from the Danish Register of Medicinal Product Statistics. Outcome measures were obtained at the first contact with the fertility clinic, and categorized according to the International Classification of Diseases (ICD-10). The association between current use of phthalate-containing medications <90 days prior to semen sampling and reduced semen quality was analysed using unconditional logistic regression, adjusting for potential confounders.

MAIN RESULTS AND THE ROLE OF CHANCE: In total, 57 cases and 72 controls redeemed at least one prescription for a drug containing ortho-phthalates in the 90 days before their first semen sample, yielding an adjusted odds ratio (OR) of 1.30 (95% CI: 0.91–1.85) for poor semen quality when compared to males exposed to phthalate-free generic drugs. Similarly, 81 cases and 78 controls exposed to a drug containing polymers had increased odds of poor semen quality (OR = 1.71, 95% CI: 1.24–2.35). Current exposure to polymer containing products from alimentary tract and metabolism drugs was associated with the highest OR of 2.80 (95% CI: 1.63–4.84). Comparing males exposed to drugs containing ortho-phthalates or polymers with males unexposed to prescription drugs, we found adjusted ORs of 1.32 (95% CI: 0.93–1.87) and 1.73 (95% CI: 1.26–2.36), respectively. We saw no clear relationship between degree of exposure and odds of poor semen quality.

LIMITATIONS, REASONS FOR CAUTION: The reliance on ICD-10 based register data restricted our ability to relate phthalate exposure to detailed semen parameters. Furthermore, due to imperfections in the registry, we could only include the first semen sample and could not follow semen quality over time.

WIDER IMPLICATIONS OF THE FINDINGS: Our results support the likely negative effect of phthalate exposure from medicinal drugs on semen quality. As exposures from medicinal products are readily avoidable, our findings may be of relevance to regulatory authorities.

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Key words: infertility / assisted reproduction / ethics / epidemiology / semen analysis / phthalate / pharmacoepidemiology

Introduction

The increasing prevalence of poor semen quality is a global problem, causing concern over male fertility (Swan *et al.*, 2000; Jørgensen *et al.*, 2012; Romero-Otero *et al.*, 2015; Centola *et al.*, 2016). Consequently, the need for ART and IUI has increased in Europe and the USA (Swan *et al.*, 2000; Jensen *et al.*, 2008; Danish Fertility Society, 2014; Kupka *et al.*, 2014).

In recent years, researchers have investigated the possible role of environmental endocrine disruptors, which are thought to affect semen quality (Bay *et al.*, 2006; Rouiller-Fabre *et al.*, 2014; Hauser *et al.*, 2015; Le Moal *et al.*, 2015). Ortho-phthalates and their metabolites are endocrine disruptors that have been shown to affect hormone signalling in animal models. Of specific interest is their anti-androgenic effects, resulting in decreased anogenital distance, hypospadias, cryptorchidism, decreased testosterone levels, decreased sperm production and infertility (Borch *et al.*, 2006; Foster, 2006; Howdeshell *et al.*, 2008). Several epidemiologic studies have shown associations between low-level environmental phthalate exposure (quantified by urinary phthalate biomarkers) and poor semen quality, suggesting similar effects in humans (Skakkebaek *et al.*, 2001; Hauser, 2008; Kay *et al.*, 2014; Axelsson *et al.*, 2015; Bloom *et al.*, 2015; Cai *et al.*, 2015). However, phthalates are rapidly metabolized and excreted in both urine and faeces. Therefore, the use of biomarkers for comparing relative exposure levels to various phthalates could be misleading (Hauser and Calafat, 2005).

Not commonly recognized, a number of oral drug formulations contain ortho-phthalates and polymers as excipients to enable timed release of the active agent (Hauser *et al.*, 2004; Hernández-Díaz *et al.*, 2009; Gallinger and Nguyen, 2013). Chronic users of ortho-phthalate containing medications have substantially higher urinary concentration of phthalate metabolites—50-fold or more—compared with the general population (Hauser *et al.*, 2004; Hernández-Díaz *et al.*, 2009). In contrast to environmental exposure, exposure from a medicinal product is in principle readily avoidable. In case of documented negative influences on human reproduction from medicinal product exposures, this could have regulatory consequences.

Danish nationwide registries, including data on phthalate content in pharmaceuticals, offer a unique opportunity to study the association between exposure to phthalates through medicinal products and selected outcomes. In this article, we investigate the association between medicinal phthalate exposure and semen quality within a cohort referred to ART.

Materials and Methods

In this case–control study, we included all Danish couples registered in the Danish IVF register (IVF-R) during the period 1 January 2006 to 31 December 2016.

Data sources and linkage

Data were obtained from four population-based registries: The IVF-R (Andersen *et al.*, 1999), the Danish National Patient Registry (DNPR) (Schmidt *et al.*, 2015), the Danish CPR registry (Schmidt *et al.*, 2014) and the Danish Register of Medicinal Product Statistics (the Prescription Registry) (Pottegård *et al.*, 2016). Data on drug excipients were retrieved from the Danish Medicines Agency and linked to data from the Prescription Registry. All data sources were linked by use of a unique personal registration number (Pedersen, 2011). For detailed registry declarations, please see Supplementary Data.

Patient material

The IVF-R contains data on all fertility treatments (including both ART and IUI) performed in Denmark in both private and public clinics. All heterosexual couples were categorized as male factor, female factor, both or idiopathic infertility based on data from both the IVF-R and the DNPR. Female infertility was based on diagnoses [defined by the 8th and 10th version of the International Classification of Diseases (ICD-8/ICD-10)] relevant for female fecundity and included: endometriosis, uterine fibroma, ovarian dysfunction and female infertility (Supplementary Data: ICD codes). Male factor infertility was based on their semen sample. Abnormal semen quality was defined by ICD-10 diagnosis N46, including aspermia, azoospermia, oligozoospermia, other male infertility and male infertility unspecified. Males with 'other male infertility' were excluded if an identified reason for infertility were identified (see exclusions). Normal semen quality was defined as either registered idiopathic infertility or a normal semen sample. Additional male data included age, and information on smoking, alcohol consumption, and BMI. Comorbidities and drug exposure relevant for male fecundity were identified in the IVF-R, the DNPR and the Prescription Registry.

Identification of cases and controls

Cases were defined as all males with reduced semen quality, regardless of any identified female factors. Restricted case definitions were employed for sensitivity analyses: males with reduced semen quality in couples without any female factors as identified in the IVF-R; and males with reduced semen quality in couples without any female factors identified in either the IVF-R or in the DNPR. Controls were identified as all males with normal semen quality who did not meet any of the exclusion criteria.

Exclusions

We excluded couples who did not live in Denmark for at least 12 months before their first fertility treatment and to ensure the date of semen sampling was the same as the date of treatment, we limited the analysis to each couple's first completed IUI or aspiration, excluding all treatments with frozen eggs. Couples referred for PGD, and couples diagnosed with HIV, hepatitis B or hepatitis C were excluded, as were males with a diagnosis of cancer (except skin cancer), retrograde ejaculation, testicular or epididymal sperm extraction, or previous sterilization. Lastly, if a male recurred with more partners in the registry, any treatments with later partners were excluded. A complete list of ICD-8 and ICD-10 codes used for exclusions appears in Supplementary Data.

Study drugs

The Danish Medicines and Health Authorities maintain an internal database with detailed information on specific marketed products, including quantifiable excipient content such as phthalates (The Danish Medicines Authority, 2017). We constructed a dataset listing all medicinal products containing phthalates marketed in Denmark between 2004 and 2016. The dataset includes the Anatomical Therapeutic Chemical (ATC) code for the active substance as defined by the Anatomical Therapeutic Chemical Classification System, the Nordic article number (VNR) for the specific product, the specific phthalate excipient (ortho-phthalates and/or polymers), and the amount used in the specific product formulation. Specific VNR codes for phthalate-containing product formulations, and phthalate-free versions of the same generic products were identified using the ATC code, and merged with prescription records from the Prescription Registry.

Exposure

Exposure was defined as having filled a prescription for a phthalate-containing product. Five different phthalates were identified and classified into two groups: ortho-phthalates: diethyl phthalate (DEP) and dibutyl phthalate (DBP); polymers: cellulose acetate phthalate (CAP), poly vinyl acetate phthalate (PVAP) and hydroxypropyl methylcellulose phthalate (HPMCP). Phthalate exposure was assumed to start on the day of prescription collection and, with the exception of systemic antibiotics, a standard treatment duration of 90 days was assumed. For antibiotics, we assigned the entire phthalate load to the prescription fill date. Three exposure windows were defined: current (1–90 days), recent (91–180 days) and former (181–365 days) use before the date of first fertility treatment per couple. Only days of exposure overlapping with the exposure window would be accounted for. For subanalyses, an 'immediate' exposure window was defined as 1–10 days prior to the first fertility treatment. Prescriptions redeemed on the day of fertility treatment were ignored. As spermatogenesis takes ~70 days (Amann, 2008), the 'current exposure' definition was of primary interest.

Statistical analyses

Using unconditional logistic regression, we calculated adjusted odds ratios (ORs) associating phthalate exposure with poor semen quality. To address confounding by indication, we compared males exposed to phthalate-containing prescription drugs to males exposed to phthalate-free versions of the same ATC groups. For minimally adjusted models we included age and year of treatment as covariates. For fully adjusted models we further included previous diagnosis of ≥1 fertility-related comorbidities (Table I), and prescription for drugs known or suspected to negatively affect male fertility (Table I). For all drugs, exposure was limited to prescriptions redeemed within 180 days before the first ART. A full list of diagnosis codes used can be found in Supplementary Data. We performed several sensitivity analyses. First, we restricted our study population to cases and controls unexposed to any of these drugs. Second, we changed the comparison group from males exposed to phthalate-free versions of the same ATC groups to males unexposed to prescription drugs.

We calculated the cumulated amount of exposure to each specific phthalate, and for each type of phthalate we calculated the range of exposure and calculated the odds of abnormal semen quality within tertiles of cumulative exposure. For current exposure, we identified males exceeding the daily maximum exposure limits for ortho-phthalates (DEP and DBP) over the entire 90-day window. The exposure limits were set at 4.0 mg/kg body weight/day for DEP, and 0.01 mg/kg body weight/day for DBP, according to European Medicines Agency guidelines (EMA, 2014; Broe *et al.*, 2017). No exposure limits have been introduced for high molecular weight polymers due to low absorption (Kossor *et al.*, 2014).

Other

Stata Version 14.2 (StataCorp, College Station, TX, USA) was used for all analyses.

Ethical approval

The study was approved by the Danish National Health Data Board (SDS) and the Danish Data Protection Agency (Jour. no. 2015-57-0008).

Table I Prevalence of selected drug exposure and diagnoses among cases and controls.

	Cases† (*n* = 18 515)	Controls (*n* = 31 063)
Age, median (IQR)	34 (30–38)	34 (30–38)
Phthalate exposure from medication in the last 90 days		
Any type	106 (0.6%)	112 (0.4%)
Phthalate polymers	81 (0.4%)	78 (0.3%)
Chronic users (%)	48.1%	42.3%
Ortho-phthalates	57 (0.3%)	72 (0.2%)
Chronic users (%)	33.3%	38.9%
Prescription drug use within the 180 days preceding ART/IUI		
Sulfasalazine	44 (0.2%)	26 (0.1%)
Spironolactone	(*n* < 5)	11 (0.0%)
Cimetidine	7 (0.0%)	7 (0.0%)
Nifedipine	13 (0.1%)	6 (0.0%)
Ketoconazole	(*n* < 5)	5 (0.0%)
Testosterone	93 (0.5%)	20 (0.1%)
Finasteride	23 (0.1%)	35 (0.1%)
SSRI	361 (1.9%)	613 (2.0%)
Alpha blockers	18 (0.1%)	40 (0.1%)
Opiates	321 (1.7%)	518 (1.7%)
Any of above drugs	887 (4.8%)	1281 (4.1%)
Previous diagnosis of*		
Cryptorchidism	1289 (7.0%)	675 (2.2%)
Varicocele	46 (0.2%)	37 (0.1%)
Inguinal hernia	399 (2.2%)	563 (1.8%)
STI	35 (0.2%)	42 (0.1%)
Testicular torsion	196 (1.1%)	215 (0.7%)
Orchitis	47 (0.3%)	58 (0.2%)
Testicular atrophy	19 (0.1%)	11 (0.0%)
Endocrine disorders	55 (0.3%)	60 (0.2%)
Genetic disorders	13 (0.1%)	12 (0.0%)
Diabetes	48 (0.3%)	49 (0.2%)
Any of above diagnoses	2147 (11.6%)	1722 (5.5%)

†All males with reduced semen quality regardless of any identified female factors.
*International Statistical Classification of Diseases (ICD)-8 and ICD-10 codes are listed in Supplementary Data 2.
IQR, interquartile range; SSRI, selective serotonin re-uptake inhibitors; STI, sexually transmitted infections.

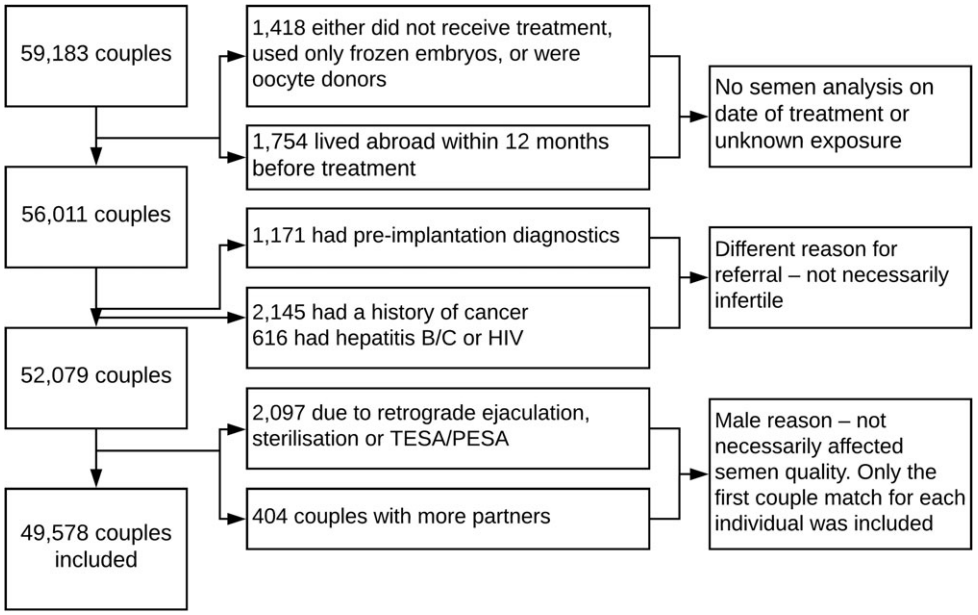

Figure 1 Flow chart presentation of study population exclusions. TESA/PESA, testicular sperm aspiration/percutaneous epididymal sperm aspiration.

According to Danish law, ethics approval is not required for register studies.

Results

We identified 59 183 unique heterosexual couples in the IVF registry between 1 January 2006 and 31 December 2016. After applying exclusions, 49 578 unique couples remained in the study (Fig. 1). The main analyses comprised a total of 18 515 cases with low semen quality (Fig. 2). For the two case definitions restricted to couples without any female factors we identified a total of 12 634 and 5091 cases, respectively. A total of 31 063 controls were identified and used to represent the at-risk population for all three case definitions.

Compared with controls, males with abnormal semen quality had a higher prevalence of fertility-related comorbidities (11.6 versus 5.5%). Adjusting for these comorbidities did not substantially affect ORs that were already adjusted for age and year of treatment. Cases and controls were similarly exposed to drugs with known or suspected effects on fertility (Table I). Selected demographic characteristics, including alcohol, smoking and BMI, were only sporadically available (Supplementary Table SI).

Table II shows the comparison of males exposed to identical active drugs in formulations with ortho-phthalates and/or polymers, and drugs without phthalates. Current exposure to any of the study products containing ortho-phthalates (upper panel) was, although statistically insignificant, associated with a 30% relative increase in the odds of poor semen quality [adjusted OR of 1.30 (95% CI: 0.91–1.85)], whereas current exposure to any of the study drugs containing polymers (lower panel) was associated with a 71% relative increase in the odds of poor semen quality [adjusted OR of 1.71 (95% CI: 1.24–2.35)]. When comparing males exposed to phthalate-free drugs to males unexposed to prescription drugs, we found an adjusted OR of 1.00 (0.94–1.07), indicating no difference in risk of poor semen quality (data not shown).

Using an exposure window of 90 days yielded an adjusted OR of 1.57 (95% CI: 1.21–2.05) for poor semen quality (Table III). When restricting to cases and controls who were unexposed to the prescription drugs listed in Table I, we found an adjusted OR of 1.36 (95% CI: 0.99–1.88) for poor semen quality (Supplementary Table SII).

No clear relationship was found between tertiles of exposure and odds of poor semen quality. Assuming a body weight of 70 kg and an evenly distributed exposure during the 90 days prior to ART, 15 cases and 17 controls exceeded the daily maximum exposure limits (DME) defined by regulatory agencies for DBP of 0.01 mg/kg/day (Supplementary Table SIII). No males exceeded the DME of 4.0 mg/kg/day set for DEP.

The highest odds for poor semen quality was found among males chronically exposed to phthalates (Table II). Results for the two case definitions restricted to couples without any female factors were presented in Supplementary Tables SIV–SVIII.

Discussion

In this unique, large case–control study we found that exposure to medicinal products containing ortho-phthalates or polymers was associated with a decreased sperm quality (OR = 1.32, 95% CI: 0.93–1.87) and (OR = 1.73, 95% CI: 1.26–2.36), respectively. The highest OR was found for exposure to polymers from alimentary tract and metabolism drugs, specifically bisacodyl and sulfasalazine. Stratification per specific phthalate did not yield an obvious pattern. Analysis of cumulative exposure did suggest some cumulative dose–response relationship based on tertile exposure, but the precision of these estimates was

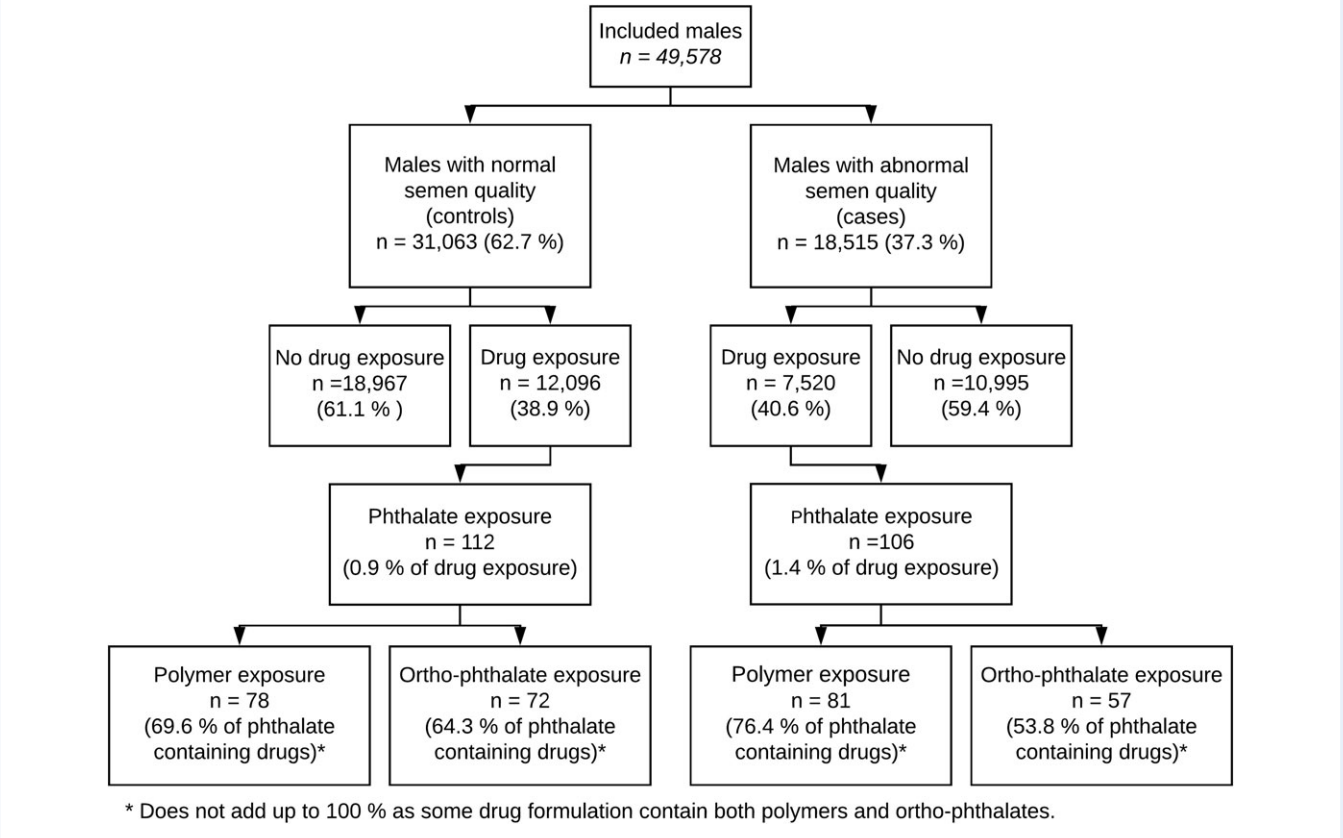

Figure 2 Main study population: drug exposure during the last 90 days prior to semen sampling among cases and controls.

poor owing to the low number of cases and controls in each stratum (Supplementary Table SIII).

Several studies have assessed the association between low-level environmental ortho-phthalate exposure during adulthood and poor semen quality. The vast majority of these comprise patients from fertility clinics, using urinary phthalate metabolite concentrations as markers of environmental exposure (Duty *et al.*, 2003; Hauser *et al.*, 2006, 2007; Wirth *et al.*, 2008; Herr *et al.*, 2009; Liu *et al.*, 2012; Jurewicz *et al.*, 2013; Han *et al.*, 2014; Wang *et al.*, 2015, 2016b; Chang *et al.*, 2017); while others relied on blood or seminal fluid for exposure characterization (Pant *et al.*, 2014; You *et al.*, 2015; Wang *et al.*, 2016a). Study subjects comprised healthy young men (Joensen *et al.*, 2012; Axelsson *et al.*, 2015), partners of pregnant women (Thurston *et al.*, 2015), or partners of females discontinuing contraception (Bloom *et al.*, 2015). Joensen *et al.* (2012), Thurston *et al.* (2015) and Bloom *et al.* (2015) had inconclusive results, while Axelsson *et al.* (2015) ound an association between phthalate exposure and poor semen quality. All of these studies were based on low environmental sources of exposure and relied fully on biomarkers collected on the date of semen sampling, and are therefore not necessarily reflecting the exposure levels during the period of spermatogenesis.

Only one other research group has evaluated the potential association between phthalate exposure from identified medical products and semen quality. Nassan *et al.* (2016) designed an interventional crossover-crossback study in males suffering from inflammatory bowel disease. During two times 4 months, patients were exposed to two different formulations of mesalamine; with and without phthalate, respectively. Males unexposed to phthalates at baseline had a decline in semen quality after crossover, and a cumulative carryover effect of the high phthalate exposure into the crossback interval (Nassan *et al.*, 2016). These observations are in accordance with our findings. For mesalazine, we demonstrate an adjusted OR of 1.70 (0.53–5.45), which, while imprecise, suggests an effect of ortho-phthalate exposure on spermatogenesis. Our results do not suggest that effects differ between ortho-phthalates and polymers. As effects on spermatogenesis are hypothesized to be mediated through anti-androgenic effects of systemically available phthalates, this observation is somewhat unexpected, as polymers are generally not believed to be systemically available to a meaningful extent (Kossor *et al.*, 2014). Our findings did suggest differences between drug classes, and between sporadic and chronic exposure to phthalate-containing drugs. Cases were more often chronic users of drugs containing polymers, and controls were more often chronic users of ortho-phthalate containing drugs. These findings require further exploration in a larger population sample.

A limitation to our study was the reliance on ICD-10 diagnoses for classification of semen quality. The main level classification is reliable, but sub stratification of semen quality may be less valid. Semen quality is further classified according to sperm concentration, amount of morphologically abnormal spermatozoa, sperm motility, and presence of sperm in ejaculate. Normal values of such parameters are mentioned in five consecutive laboratory manuals published by the World Health Organization since 1980 (WHO, 2010). In the 2010 edition, the limit

Table II Cases and controls exposed to drugs containing ortho-phthalates (upper panel) or phthalate polymers (lower panel) compared to cases and controls exposed to phthalate-free drugs within the same Anatomical Therapeutic Chemical group. Limited to generic drugs with more than five cases exposed to the phthalate-containing version.

90 Days before index date**	Cases† Phthalate containing/ free	Controls	Chronic users %***	Crude OR	Adjusted OR*
Ortho-phthalates					
Any ATC code	57/1421	72/2370		1.32 (0.93–1.88)	1.30 (0.91–1.85)
A—ALIMENTARY TRACT AND METABOLISM	15/136	15/214	(53/47)	1.57 (0.75–3.32)	1.57 (0.74–3.32)
J—ANTIINFECTIVES FOR SYSTEMIC USE	15/42	24/69	(0/ 8)	1.03 (0.48–2.17)	0.92 (0.42–2.02)
M—MUSCULO-SKELETAL SYSTEM	16/1202	24/2034	(13/8)	1.13 (0.60–2.13)	1.10 (0.58–2.08)
N—NERVOUS SYSTEM	10/67	8/85	(60/88)	1.59 (0.59–4.24)	1.62 (0.60–4.39)
A06AB02 Bisacodyl	7/6	5/<5	(29/20)	0.70 (0.12–4.23)	0.36 (0.04–3.17)
A07EC02 Mesalazin	7/45	10/95	(71/60)	1.48 (0.53–4.13)	1.70 (0.53–5.45)
J01FA01 Erythromycin	15/19	24/23	(0/8)	0.76 (0.31–1.84)	0.79 (0.32–1.95)
M01AB05 Diclofenac	6/208	7/329	(33/0)	1.36 (0.45–4.09)	1.28 (0.42–3.88)
M01AB55 Diclofenac, combinations	5/8	6/10	(0/17)	1.04 (0.23–4.70)	1.15 (0.23–5.92)
N05AN01 Lithium	8/11	7/10	(75/86)	1.04 (0.28–3.92)	1.07 (0.27–4.24)
Phthalate polymers					
Any ATC code	81/1421	78/2370		1.73 (1.26–2.38)	1.71 (1.24–2.35)
A—ALIMENTARY TRACT AND METABOLISM	43/136	24/214	(60/58)	2.82 (1.64–4.86)	2.80 (1.63–4.84)
J—ANTIINFECTIVES FOR SYSTEMIC USE	15/42	25/69	(0/8)	0.99 (0.47–2.08)	0.89 (0.41–1.94)
M—MUSCULO-SKELETAL SYSTEM	6/1202	9/2034	(0/11)	1.13 (0.40–3.18)	1.12 (0.40–3.17)
N—NERVOUS SYSTEM	9/ 67	8/85	(67/75)	1.43 (0.52–3.90)	1.43 (0.52–3.92)
A06AB02 Bisacodyl	8/6	5/<5	(25/20)	0.80 (0.13–4.74)	0.37 (0.04–3.35)
A07EC01 Sulfasalazin	29/15	16/9	(66/69)	1.09 (0.39–3.04)	1.05 (0.37–2.96)
J01FA01 Erythromycin	15/19	24/23	(0/8)	0.76 (0.31–1.84)	0.79 (0.32–1.95)
N03AG01 Valproic acid	7/29	5/38	(71/100)	1.83 (0.53–6.37)	1.69 (0.47–6.12)

†All males with reduced semen quality regardless of any identified female factors.
*Adjusted for age and index year.
**Includes cases and controls exposed at any point within 90 days prior to ART.
OR, odds ratio; ATC, Anatomical Therapeutic Chemical.
***% Chronic users (cases/controls) defined as at least three prescriptions within the last 12 months containing either ortho-phthalates (upper panel) or phthalate polymers (lower panel).

of normal sperm concentration was changed from 20 million/mL to 15 million/mL. Using the ICD-10 codes in Denmark it is not possible to code reduced sperm motility, as this code does not exist, and it is also not possible to classify abnormal morphology without decrease in concentration. The IVF-R is limited to couples attending a fertility clinic, and public referral is restricted to couples unable to conceive after 12 months, unless any obvious cause of infertility is present in either partner. However, we have no reason to believe that phthalate exposure is related to the decision of fertility treatment or not. When identifying the control group, we assumed that a normal semen sample would imply normal fertility. However, it is possible that phthalate exposure can affect unmeasured functionality of semen.

To address confounding by indication or active drug ingredients in the prescription drug used to treat their condition, we compared phthalate-exposed individuals with males using the same generic drug but in a phthalate-free version. No such confounding was apparent and, somewhat puzzling, we found a null effect when comparing males exposed to phthalate-free drugs to males unexposed to prescription drugs. The Danish reimbursement system in effect is based on the cheapest generic drug. Pharmacies are required by law to offer the patient the cheapest generic version, and phthalate content is unrelated to the product price. For the specific ATC codes, the signal was persistent for alimentary tract and metabolism drugs, while analysis for confounding mitigated the apparent signal from drugs belonging to anti-infectives for systemic use, musculo-skeletal system and nervous system groups. The subgroup analysis is compromised by the low number of cases and controls exposed to the specific individual drug with and without phthalate (Table II). Furthermore, the sample size did not permit meaningful exploration of specific phthalate exposure and specific semen parameters. Phthalate exposure from environmental sources is unaccounted for. However, the estimated level of exposure from environmental sources is several orders of magnitude lower than quantifiable exposure from drugs, and unlikely to substantially influence our analysis (Hernández-Díaz *et al.*, 2009; Hait *et al.*, 2014). The analysis comparing exposure to identical active moieties with and without phthalate would also mitigate the influence of environmental exposure.

In conclusion, exposure to drugs containing ortho-phthalates and/or polymers within 90 days prior to semen sampling was associated

Table III Exposure to phthalate-containing drugs. Immediate, current, recent and former drug use was defined as exposure to at least one phthalate-containing drug within the last 10 (immediate), 90 (current), 91–180 (recent) and 181–365 (past) days, respectively.

	Cases†	Controls	Crude OR	Adjusted* OR
Total	18 515	31 063		
Unexposed	18 258	30 766	Reference	Reference
Exposed (Any type)				
Usage				
Immediate	64	63	1.71 (1.21–2.43)	1.68 (1.19–2.39)
Current	106	112	1.59 (1.22–2.08)	1.57 (1.21–2.05)
Recent	42	64	1.11 (0.75–1.63)	1.09 (0.74–1.61)
Past	109	121	1.52 (1.17–1.97)	1.50 (1.15–1.94)
Exposed (ortho-phthalates)				
Usage				
Immediate	28	32	1.47 (0.89–2.45)	1.45 (0.87–2.41)
Current	57	72	1.33 (0.94–1.89)	1.32 (0.93–1.87)
Recent	32	51	1.06 (0.68–1.65)	1.05 (0.67–1.63)
Past	92	112	1.38 (1.05–1.82)	1.37 (1.04–1.80)
Exposed (polymers)				
Usage				
Immediate	49	41	2.01 (1.33–3.05)	1.98 (1.31–3.00)
Current	81	78	1.75 (1.28–2.39)	1.73 (1.26–2.36)
Recent	33	51	1.09 (0.70–1.69)	1.08 (0.69–1.67)
Past	77	76	1.71 (1.24–2.35)	1.69 (1.23–2.33)
Current exposure to phthalates				
Both ortho-phthalates and polymers	32	38	1.42 (0.89–2.27)	1.40 (0.88–2.25)
Ortho-phthalates (no polymers)	25	34	1.24 (0.74–2.08)	1.22 (0.73–2.05)
Polymers (no ortho-phthalates)	49	40	2.06 (1.36–3.14)	2.03 (1.34–3.09)
DEP	35	48	1.23 (0.79–1.90)	1.22 (0.79–1.88)
DBP	22	24	1.54 (0.87–2.76)	1.52 (0.85–2.71)
PVAP	7	5	2.36 (0.75–7.43)	2.33 (0.74–7.35)
HPMCP	35	43	1.37 (0.88–2.14)	1.35 (0.86–2.11)
CAP	40	30	2.25 (1.40–3.61)	2.23 (1.39–3.58)

†All males with reduced semen quality regardless of any identified female factors.
*Adjusted for age and index year.
DEP, diethyl phthalate; DBP, dibutyl phthalate; PVAP, poly vinyl acetate phthalate; HPMCP, hydroxypropyl methylcellulose phthalate; CAP, cellulose acetate phthalate.

with a 30 and 71% increased risk of poor semen quality, respectively. Our findings support a likely negative effect of phthalates exposure on semen quality, and may be of relevance to the regulatory authorities.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

A.B. and A.P. had access to all data used in this study and take full responsibility of the accuracy of the data acquisition and analyses. P. D., J.H., A.P., T.A. and A.B. were responsible for the study design. A. B. drafted the article, but analysis, interpretation and critical revision of the final manuscript were conducted by all authors.

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Conflict of interest

None of the authors declare conflict of interest.

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