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Male Urinary Paracetamol and Semen Quality

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Summary

The endocrine disrupting properties of paracetamol have been previously demonstrated in rodent students of abnormal sperm morphology and diminished testosterone production; in addition to epidemiologic studies of diminished couple fecundity. In this study, we examined the relationship between paracetamol and its metabolite *p*-aminophenol quantified in a single spot urine and semen quality among 501 male partners of couples planning for pregnancy. Men provided a urine specimen and two fresh semen samples collected approximately one month apart and underwent 24-hour analysis for 35 semen quality parameters. Paracetamol and *p*-aminophenol were quantified in urine by ultra-high performance liquid chromatography coupled with an electrospray triple quadrupole mass spectrometry. The relationship between natural log transformed urinary paracetamol and *p*-aminophenol rescaled by their standard deviation and 21 Box-Cox transformed, 14 non-transformed semen parameters was assessed using linear mixed-effects models. The median concentrations (IQR) of urinary paracetamol and *p*-aminophenol were 15.5 ng/mL (5.44, 73.5) and 978 ng/mL (500, 1596), respectively. Following adjustment for creatinine and age, a 1-standard deviation increase in log-transformed urinary paracetamol was associated with a reduction in beat cross frequency and an increase in DNA fragmentation[β(95% CI): −0.59

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Authors' Contributions

M.M.S. assisted in conceptualizing the analytic plan and paper, and wrote the first draft. K.K. contributed to the acquisition of data, assisted in interpretation of the data, and critical revision of the paper. Z.C. oversaw the statistical analysis, and critically discussed and revised the paper. S.K. oversaw the statistical analysis, and critically discussed and revised the paper. G.M.B.L. designed the study, conceptualized the paper, assisted in interpretation of the data, and provided critical revision of the paper.

Conflict of interest

The authors have no conflicts of interest to declare.

Hz (−1.16, −0.03) and 0.05 % (0.01, 0.09), respectively]. These findings were corroborated in models of categorical chemical concentrations; higher concentrations of paracetamol remained associated with reduced beat cross frequency and increased DNA fragmentation. A 1-standard deviation increase in log-transformed urinary *p*-aminophenol was associated with a reduction in sperm head area [β (95% CI): −0.1 (−0.18, −0.02) and width −0.02 (−0.04, −0.01)]. However, only the association with sperm head area remained statistically significant in models of *p*-aminophenol quartiles. Our findings suggest that adult male urinary paracetamol is associated with sperm motility and DNA fragmentation, while the metabolite, *p*-aminophenol, is predominantly associated with sperm head morphometry.

Introduction

Paracetamol, also known as acetaminophen, is found in a large number of over-the-counter (OTC) and prescription drugs given its antipyretic and analgesic properties (Lank 2014). Having a short biologic half-life, 85%-95% of the chemical is excreted by the kidneys within 24 hours post-ingestion exposure (Forrest *et al.* 1982). Both paracetamol and its primary metabolite, *p*-aminophenol, are also major metabolites of the environmentally ubiquitous chemical aniline. A chemical used in the synthesis and manufacturing of many industrial products (e.g., dyes (indigo), polyurethane foam) (Agency for Toxic Substances and Disease Registry 2011), aniline is rapidly metabolized and excreted as both *p*-aminophenol and paracetamol. Biomonitoring data of Danish and German populations suggest sustained levels of paracetamol in the body as the result of aniline exposure (Dierkes *et al.* 2014; Modick *et al.* 2014). Common use of paracetamol/acetaminophen in conjunction with the omnipresence of environmental aniline may result in passive and possibly continuous exposure and low-level concentrations in the body (Holm *et al.* 2015).

The endocrine disrupting properties of paracetamol have been previously demonstrated in rodent fetal studies of increased percentage of abnormal sperm head, reduced sperm count and impaired motility (Ekaluo *et al.* 2008; Ratnasooriya & Jayakody 2000); reduced anogential distance and decreased testosterone production (Kristensen *et al.* 2011; Kristensen *et al.* 2012; Thiele *et al.* 2013), and epidemiologic research focusing on environmentally relevant concentrations of *in utero* paracetamol exposure and male reproductive disorders (Jensen *et al.* 2010; Philippat *et al.* 2011; Snijder *et al.* 2012). Moreover, a previous *in vitro* study with adult human testis cells reported a reduction in testosterone secretion by 18% and 30%, after 24 hours, in response to paracetamol exposure at 10^{-5} and 10^{-4} molar concentrations, respectively (Albert *et al.* 2013). The implications of changes in testosterone levels in the adult male on semen quality (e.g. sperm count) and overall fertility have long been established. In a previous analysis of the Longitudinal Investigation of Fertility and the Environment (LIFE) Study cohort, we found higher concentrations of male urinary paracetamol to be associated with a longer time-to-pregnancy for couples (Smarr *et al.* 2016). Nonetheless, epidemiologic research of adult male paracetamol exposure and male reproductive health are scarce. Therefore, we examined the relationship between paracetamol and *p*-aminophenol quantified in urine and semen quality among 501 male partners of couples planning for pregnancy.

Materials and Methods

Study population and participants

Male partners were recruited for participation in the LIFE Study, a prospective cohort of 501 reproductive aged couples from16 counties in Michigan and Texas between 2005 and 2009 who were recruited upon discontinuing contraception for ≤ 2 months prior to enrollment for purposes of becoming pregnant (Buck Louis *et al.* 2011). Inclusion criteria were: male partners needed to be ≥18 years of age; in a committed relationship; no physician diagnosis of sterility; and an ability to communicate in English or Spanish. Institutional review board approvals were obtained from all collaborating institutions; couples gave written informed consent prior to study participation and any data collection.

Data and Biospecimen collection

Upon enrollment, in-person interviews were conducted with male partners to ascertain lifestyle and reproductive history followed by standard anthropometric assessments to measure body mass index (BMI) (Lohman *et al.* 1988). During the enrollment home visit, male partners provided a nonfasting baseline urine sample. Additionally,, male partners provided a baseline semen sample, followed by a second semen sample, approximately 1 month apart. Following a 2-day abstinence period after enrollment, males obtained specimens via masturbation, without the use of lubricants, using at-home collection kits (Royster *et al.* 2000). Collection kits included a glass collection jar with an attached button thermometer to monitor temperature every half hour throughout the process, a glass sperm migration straw (Vitrotubes 3520; VitroCom Inc., Mountain Lakes, NJ) containing hyaluronic acid and plugged at one end, and cold packing materials for shipping. Male partners were instructed to collect the semen sample in the jar, place the sperm migration straw into the jar (as an exploratory marker of sperm motility and viability at the time of collection), and to record on the label the time of last ejaculation and any spillage. Specimens were shipped in insulated shipping containers containing ice packs overnight via Federal Express to the andrology laboratory at the National Institute for Occupational Safety and Health (Cincinnati, OH). Men were instructed to call a toll-free hotline to report the shipment of their semen samples. Semen delivered to a central andrology laboratory by overnight mail in insulated mailing kits have been successful in maintaining specimens for other studies (Luben *et al.* 2007; Olshan *et al.* 2007; Royster *et al.* 2000).

Semen analysis

Following overnight delivery of semen samples, the 24-hour semen analysis comprised inspecting sample integrity and that shipment temperatures were within established ranges (via temperature logging monitor, Maxim Integrated, San Jose, CA, USA), along with general characteristics including turbidity, color, liquefaction, and volume. Samples were then warmed to 37°C and volume was measured to the nearest 0.1 cc. Established laboratory protocols that included ongoing quality assurance and control procedures (American Society of Andrology, 1996). Semen analysis after home collection has been reported to be reliable for all semen parameters with the exception of motility parameters (Stovall *et al.* 1994). A percentage of sperm are alive after 24 hours and a next-day motility assessment still can be made and may provide important information on sperm function and survivability (Stovall

et al. 1994). A total of 35 semen parameters (5 general characteristics: sperm concentration, volume, total count, straw distance, hypo-osmotic swollen; 8 motility measures; 6 sperm head measures; 12 individual and 2 summary morphology measures; and 2 sperm chromatin stability measures)were quantified.. Six parameters are derived from other parameters. Specifically, sperm concentration is equal to total sperm count divided by volume; sperm head area, perimeter, and elongation factor are functions of sperm head length and width; percent linearity is a function of the straight-line and curvilinear velocity; percent straightness is a function of straight-line and average path velocity.

Sperm concentration was assessed using the IVOS system and the IDENT™ stain (all from Hamilton Thorne Biosciences, Beverly, MA)(Zinaman *et al.* 1996). A migration straw was used so the lab could microscopically assess the distance the vanguard sperm traveled to the nearest millimeter, which indicated sperm motility at the time of collection, in light of using next-day analysis (Turner & Schrader 2006) Sperm viability was determined by hypo-osmotic swelling (HOS assay)(Jeyendran *et al.* 1992; Schrader *et al.* 1990) and sperm motility was assessed using the HTM-IVOS computer assisted semen analysis system (CASA) microscope slides were prepared for sperm morphometry. Morphology was assessed via slides prepared by Fertility Solutions® (Cleveland, OH) and was assessed using both traditional (World Health Organization 1992) and strict (Rothmann *et al.* 2013) classifications. Sperm morphometry was conducted using the IVOS METRIX system (Hamilton Thorne Biosciences). One aliquot of whole semen was diluted in TNE buffer and frozen for the sperm chromatin stability assay (SCSA) (Evenson *et al.* 2002). SCSA® analysis was conducted by SCSA Diagnostics (Brookings, SD) using a Coulter Epics Elite Flow Cytometer (Coulter, Miami, FL). The SCSA® assay measures sperm DNA damage, which is then quantified as the percentage of separated or damaged DNA (DNA fragmentation index; DFI) and the percentage of highly immature sperm nuclei with abnormal proteins (high stainability)(Evenson 2013).

The second semen sample was assessed to corroborate azoospermia observed in the first sample; in the event of such an observation males were advised to seek clinical care. An abbreviated semen analysis was performed on the second sample (i.e., volume, concentration, next-day motility, and sperm head morphology).

Urinary analysis of chemicals

Chemical analysis of total urinary paracetamol and *p*-aminophenol was performed at the Wadsworth Center, New York State Department of Health, Albany, NY. Specifically, 300 μL of 1 M ammonium acetate containing 30 U of β-glucuronidase (pH=5.5) was added to 500 μL of urine sample, followed by incubation at 37°C for 12 h. Target analytes were extracted thrice with ethyl acetate and were quantified by ultra-high performance liquid chromatography (Acquity I Class; Waters, Milford, MA) coupled with an electrospray triple quadrupole mass spectrometry (API 5500; AB SCIEX, Framingham, MA) (UPLC-ESI-MS/MS). Separation of target analytes was carried by a Kinetex C18 (1.3 μ, 100A, 50 × 2.1 mm) column (Phenomenex; Torrance, CA) with a SecurityGuard guard column (Phenomenex) with positive ionization, multiple reaction monitoring mode of detection. Quality assurance and quality control parameters included procedural blanks, matrix spikes

and duplicate analysis of samples. Labelled internal standards (d_7-*p*-aminophenol and $^{13}C_2$-^{15}N- paracetamol) were spiked into all samples and quantification was by isotope dilution. The method limits of quantitation (LOQ) for *p*-aminophenol and paracetamol were 0.25 and 0.5 ng/mL, respectively.

Statistical analysis

Five (1%) men were azoospermic on both samples and excluded from analysis. Univariate analyses assessed the distributions of paracetamol, *p*-aminophenol and relevant covariates. Male lifestyle characteristics were compared by quartiles of urinary paracetamol using Chi-Square and Wilcoxon non-parametric tests for categorical and continuous covariates. Median and accompanying interquartile ranges (IQRs) of baseline urinary paracetamol concentrations (ng/mL) were calculated; medians were compared between males who did/did not provide a semen sample using Wilcoxon-Mann-Whitney tests.

To avoid biasing regression estimates, instrument-derived values paracetamol were used in all models with urinary creatinine included as a covariate; urinary concentrations below the LOQ were not substituted in any manner (Lubin *et al.* 2004; Richardson & Ciampi 2003; Schisterman *et al.* 2006). Urinary creatinine, paracetamol, and *p*-aminophenol concentrations were natural-log transformed (ln) to normalize distributions; paracetamol and *p*-aminophenol concentrations were also rescaled by their standard deviations for a meaningful interpretation of regression estimates. Furthermore, 21 of the 35 semen parameters underwent Box-Cox transformations to approach approximately normal distributions prior to regression analyses. Specifically, 14 semen end points required ln transformation; 6 underwent cubic root transformation; and 1 end point, % strict criteria, was required a seventh root transformation.

In linear mixed effects models, accounting for dependence between the two semen samples, urinary concentrations of paracetamol were modeled individually for each semen parameter and adjusted based on *a priori* selection of covariates: non-time varying age (years) and urinary creatinine (ng/mL). To assess potential non-linear associations, models were performed with both exposures categorized into quartiles. Wald tests were performed to test for nonlinear trend across quartiles of chemical concentrations. Linear and quartile models of summed concentrations of paracetamol and *p*-aminophenol were also performed given their low correlation (Spearman's $r^2 = 0.09$, p=0.08), which may be indicative of variation in exposure source. All analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC).

Results

Semen samples were obtained for 468 (93%) non-azoospermic males, urinary chemicals were quantified for 439 (88%); for a total of 414 (83%) male partners with semen and paracetamol data. The average age and body mass index of male partners was 31.8 (SD=4.8) years and 30 (SD=5.7) kg/m^2, respectively (Table 1). Males in the present analysis were predominantly overweight, non-Hispanic whites, between 30–40 years of age, with household incomes between $50,000 and $99,000. Socio-demographics of male partners did not tend to differ by quartiles of urinary paracetamol and *p*-aminophenol concentrations;

comparisons by paracetamol quartiles are presented in Table 1. Furthermore, males who did not provide a semen sample were similar to those included in this analysis.

The distributions s of urinary paracetamol and *p*-aminophenol for male partners are displayed in Table 2. Overall, paracetamol and *p*-aminophenol were readily detected in male urine (93% and 100%, respectively).

Significant associations between continuous and quartile models of urinary paracetamol and semen parameters are presented in Table 3. In crude models, increasing urinary paracetamol concentration was associated with average path velocity (μm/sec), straight-line velocity (μm/sec), curvilinear velocity (μm/sec), beat cross frequency (Hz), straightness (%), and % linear movement. Following adjustment for creatinine and age, only the association with beat cross frequency (Hz) β= −0.59 (95% CI: −1.16, −0.03) remained. Additionally, a 1-SD increase in log-transformed urinary paracetamol was associated with a 0.05% increase in DNA fragmentation (95% CI: 0.01, 0.09). In non-linear models, the second quartile (5.45 – 15.96 ng/mL) of male urinary paracetamol was associated with reduced volume β= −0.19 ml, (95% CI: −0.35, −0.03) and also increased sperm count (* 10^6/ml) β= 0.87 (95% CI: 0.29, 1.46) compared with the lowest quartile (<5.44 ng/ml) after adjustment for age and creatinine. Urinary paracetamol concentrations in the third versus first quartile (16.1 – 74.6 ng/mL) were associated with reductions in beat cross frequency β= −2.13 Hz, (95% CI: −3.74, −0.52), straight motility β=−5.33 % (95% CI: −9.68, −0.99) and % micro head β= −0.16 (95% CI: −0.3, −0.03). Urinary concentrations of paracetamol in the fourth versus first quartile were associated with increased DNA fragmentation β= 0.15 % (95% CI: 0.03, 0.27) (Table 3). Associations between urinary paracetamol concentrations and all 35 semen end points, irrespective of statistical significance are reported in Supplemental Table 1.

Few significant associations were observed between urinary *p*-aminophenol concentrations and semen parameters (Table 4). Prior to model adjustment for confounders, a 1-SD increase in log-transformed *p*-aminophenol concentration was associated with reductions in sperm head [area: β = −0.08 μm^2 (95% CI: −0.14, −0.02), width: β = −0.01 μm (95% CI: −0.03, −0.002) and perimeter: β = −0.04 μm (95% CI: −0.08, −0.002). Following adjustment for age and urinary creatinine, the significant associations for sperm head area and sperm head width remained [β = −0.10 μm^2 (95% CI: −0.18, −0.02) and β = −0.02 μm (95% CI: −0.04, −0.01), respectively]. When urinary *p*-aminophenol concentrations were modeled as quartiles, the second (493 – 969 ng/mL) versus first (<491 ng/mL) quartile was associated with increased average path velocity β = 2.93 μm/sec (95% CI: 0.09, 5.78) and straight-line velocity β = 2.39 μm/sec (95% CI: 0.04, 4.75) after adjustment for age and creatinine. Positive associations were observed between the 2nd and 3rd quartiles of *p*-aminophenol and linear motility [β = 3.82 % (95% CI: 0.83, 6.82) and β = 3.19 % (95% CI: 0.14, 6.24), respectively]. Negative associations were observed between the 4th versus 1st quartile and area of sperm head (β = −0.22 μm^2 (95% CI: −0.43, −0.02)), in adjusted models. Associations between urinary p-aminophenol concentrations and all 35 semen end points, irrespective of statistical significance are reported in Supplemental Table 1.

A significant association was only observed between 1-SD increase in log-transformed summed concentrations of urinary paracetamol and *p*-aminophenol and percent motility (β =

−0.21 % (95% CI: −0.42, −0.01), prior to model adjustment; no other signals of association were observed for semen parameters, irrespective of linear model adjustment (Table 5). In models of the sum of urinary paracetamol metabolites categorized as quartiles, the third quartile (1114 – 2029 ng/mL) compared with the first (< 606 ng/mL) was negatively associated with amplitude of lateral head (μm), β = −0.37 (95% CI: −0.68, −0.06) and β = −0.43 (95% CI: −0.77, −0.09) in crude and adjusted models, respectively. Prior to, and after model adjustment, compared with the first quartile, the second quartile (606 – 1113 ng) was associated with a reduction in the percentage of other sperm tail abnormalities β = −0.20 (95% CI: −0.36, −0.05) and β = −0.22 (95% CI: −0.39, −0.05). Additionally, previous findings of increased average path velocity and percent linearity with the second quartile of *p*-aminophenol concentration (Table 4), were corroborated when paracetamol and *p*-aminophenol concentrations were summed. Specifically, the second versus first (<491 quartile was associated with increased average path velocity β = 2.87 μm/sec (95% CI: 0.07, 5.68) and percent linear motility β = 3.13 % (95% CI: 0.16, 6.09), in models adjusted for creatinine and age. Associations between the summed concentrations of urinary paracetamol and *p*-aminophenol and all 35 semen end points, irrespective of statistical significance are reported in Supplemental Table 2.

Discussion

These are the first data known to us that have assessed preconception urinary concentrations of total paracetamol and its metabolite, *p*-aminophenol, in relation to semen quality that may have further implications for couple fertility. In this exploratory analysis of 35 semen parameters, a total of 11 (31%) semen parameters were associated with increasing paracetamol concentrations and 7 (20%) with increasing concentrations of its metabolite, *p*-aminophenol, in (un)adjusted linear and non-linear regression models. Overall, we found increasing concentrations of urinary paracetamol to be associated with reductions in sperm volume, motility, and sperm head morphometry and a slight increase in DNA fragmentation. Moreover, increasing concentrations of urinary p-aminophenol tended to be associated with reductions in sperm head morphometry; lower concentrations of p-aminophenol were associated with enhanced sperm motility. When summing urinary paracetamol metabolites, findings of enhanced sperm motility were corroborated. Specifically, increased average path velocity and percent linearity.

In the context of such novel findings, consideration of biologic plausibility is essential for interpretation. One possible explanation includes the possibility that the androgenic properties of paracetamol result in reduced testosterone levels, as reported in an *in vitro* analysis of analgesics and the adult human testis (Albert *et al.* 2013). At varying molar concentrations of paracetamol, testosterone secretion was reduced by 18%-30% after 24 hours; such changes in testosterone have implications for spermatogenesis given that high levels of intra-testicular testosterone are required for during this process (Dohle *et al.* 2003). Our findings corroborate such a relationship as the lowest quartile of urinary paracetamol was associated with increased sperm count.

Interpretation of our findings in relation to other reproductive age male populations is constrained by the lack of epidemiologic assessments of urinary paracetamol or *p*-

aminophenol and semen quality. Still, the findings from the present analysis are strengthened by the home collection of two semen samples from male study participants for 24-hour semen analysis. Nonetheless, while we acknowledge that a sperm motility assessment can be made after 24 hours, we are also aware of the lack of evidence to support the reliability of sperm motility assessed after 24 hours (Stovall *et al.*1994). An additional strength of the present analysis is the biomarker quantification of paracetamol and its metabolite *p*-aminophenol that reduces the chance for misclassification on exposure. In LIFE, information on sources of potential paracetamol exposure (e.g., self-report of medication use, occupational or environmental exposures) was not collected. Furthermore, the abundance of paracetamol in contemporary environments beyond medication usage supports quantifying this exposure rather than relying on self -reported medication use to avoid bias. However, we readily acknowledge that a single spot urine collected at study baseline to estimate preconception exposures of paracetamol and its metabolite to be a potential limitation if they are not reflective of those during spermatogenesis. Nonetheless, the generalizability of our findings is supported by the high detection frequency of paracetamol (93%) in our cohort of men from Michigan and Texas, two geographically different states. Furthermore, the common use of paracetamol in medications (Lank 2014) in reproductive aged men and the assumed continuous, passive human exposure to aniline which is used in the manufacturing of various industrial products (Dierkes *et al.*2014) and rapidly metabolized into both paracetamol and *p*-aminophenol, speak to the clinical and public health relevance of such findings. Nevertheless, our findings need to be cautiously interpreted until corroborated by future research based on serial measurements of urinary paracetamol.

In conclusion, our findings demonstrate an association between urinary concentrations of paracetamol and metabolite, *p*-aminophenol and changes in semen quality among reproductive age male partners of couples trying for pregnancy. The widespread exposure and implications for couple fertility warrant future research to further elucidate mechanisms by which adult male paracetamol exposure affects semen quality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Albert O, Desdoits-Lethimonier C, Lesne L, Legrand A, Guille F, Bensalah K, Dejucq-Rainsford N & Jegou B. (2013) Paracetamol, aspirin and indomethacin display endocrine disrupting properties in the adult human testis in vitro. Human reproduction 28, 1890–1898. [PubMed: 23670170]

Andrology A S o. (1996) Semen Analysis: Challenges of QC and New Technology

Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, Boyd Barr D, Schrader SM, Kim S, Chen Z & Sundaram R. (2011) Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development--the LIFE Study. Paediatric and perinatal epidemiology 25, 413–424. [PubMed: 21819423]

Dierkes G, Weiss T, Modick H, Käfferlein HU, Brüning T & Koch HM. (2014) N-Acetyl-4-aminophenol (paracetamol), N-acetyl-2-aminophenol and acetanilide in urine samples from the general population, individuals exposed to aniline and paracetamol users. International journal of hygiene and environmental health 217, 592–599. [PubMed: 24370547]

Dohle GR, Smit M & Weber RFA. (2003) Androgens and male fertility. World Journal of Urology 21, 341–345. [PubMed: 14566423]

Evenson DP. (2013) Sperm chromatin structure assay (SCSA(R)). Methods in molecular biology (Clifton, N.J.) 927, 147–164.

Evenson DP, Larson KL & Jost LK. (2002) Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23, 25–43. [PubMed: 11780920]

Forrest JA, Clements JA & Prescott LF. (1982) Clinical pharmacokinetics of paracetamol. Clinical pharmacokinetics 7, 93–107. [PubMed: 7039926]

Holm JB, Chalmey C, Modick H, Jensen LS, Dierkes G, Weiss T, Jensen BA, Norregard MM, Borkowski K, Styrishave B, Martin Koch H, Mazaud-Guittot S, Jegou B, Kristiansen K & Kristensen DM. (2015) Aniline Is Rapidly Converted Into Paracetamol Impairing Male Reproductive Development. Toxicological sciences : an official journal of the Society of Toxicology 148, 288–298. [PubMed: 26259604]

Jensen MS, Rebordosa C, Thulstrup AM, Toft G, Sorensen HT, Bonde JP, Henriksen TB & Olsen J. (2010) Maternal use of acetaminophen, ibuprofen, and acetylsalicylic acid during pregnancy and risk of cryptorchidism. Epidemiology 21, 779–785. [PubMed: 20805751]

Jeyendran RS, Van der Ven HH & Zaneveld LJ. (1992) The hypoosmotic swelling test: an update. Archives of andrology 29, 105–116. [PubMed: 1456832]

Kilcoyne KR, Smith LB, Atanassova N, Macpherson S, McKinnell C, van den Driesche S, Jobling MS, Chambers TJ, De Gendt K, Verhoeven G, O'Hara L, Platts S, Renato de Franca L, Lara NL, Anderson RA & Sharpe RM. (2014) Fetal programming of adult Leydig cell function by androgenic effects on stem/progenitor cells. Proceedings of the National Academy of Sciences of the United States of America 111, E1924–1932. [PubMed: 24753613]

Kristensen DM, Hass U, Lesne L, Lottrup G, Jacobsen PR, Desdoits-Lethimonier C, Boberg J, Petersen JH, Toppari J, Jensen TK, Brunak S, Skakkebaek NE, Nellemann C, Main KM, Jegou B & Leffers H. (2011) Intrauterine exposure to mild analgesics is a risk factor for development of male reproductive disorders in human and rat. Human reproduction 26, 235–244. [PubMed: 21059752]

Kristensen DM, Lesne L, Le Fol V, Desdoits-Lethimonier C, Dejucq-Rainsford N, Leffers H & Jegou B. (2012) Paracetamol (acetaminophen), aspirin (acetylsalicylic acid) and indomethacin are anti-androgenic in the rat foetal testis. International journal of andrology 35, 377–384. [PubMed: 22612476]

Lank P (2014) Acetaminophen. American College of Medical Toxicology

Lohman TG, Roche AF & Martorell R (1988) Anthropometric standardization reference manual, p. Human Kinetics Books, Champaign, IL.

Luben TJ, Olshan AF, Herring AH, Jeffay S, Strader L, Buus RM, Chan RL, Savitz DA, Singer PC, Weinberg HS & Perreault SD. (2007) The healthy men study: an evaluation of exposure to disinfection by-products in tap water and sperm quality. Environmental health perspectives 115, 1169–1176. [PubMed: 17687443]

Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L & Hartge P. (2004) Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. Environmental health perspectives 112, 1691–1696. [PubMed: 15579415]

Modick H, Weiss T, Dierkes G, Bruning T & Koch HM. (2014) Ubiquitous presence of paracetamol in human urine: sources and implications. Reproduction 147, R105–117. [PubMed: 24451225]

Olshan AF, Perreault SD, Bradley L, Buus RM, Strader LF, Jeffay SC, Lansdell L, Savitz DA & Herring A. (2007) The healthy men study: design and recruitment considerations for environmental epidemiologic studies in male reproductive health. Fertility and sterility 87, 554–564. [PubMed: 17140573]

Organization) W W H. (1992) Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction

Philippat C, Giorgis-Allemand L, Chevrier C, Cordier S, Jegou B, Charles MA & Slama R. (2011) Analgesics during pregnancy and undescended testis. Epidemiology 22, 747–749. [PubMed: 21811116]

Registry A f T S a D. (2011) Toxic Substances Portal

Richardson DB & Ciampi A (2003) Effects of exposure measurement error when an exposure variable is constrained by a lower limit. American journal of epidemiology 157, 355–363. [PubMed: 12578806]

Rothmann SA, Bort AM, Quigley J & Pillow R. (2013) Sperm morphology classification: a rational method for schemes adopted by the world health organization. Methods in molecular biology (Clifton, N.J.) 927, 27–37.

Royster MO, Lobdell DT, Mendola P, Perreault SD, Selevan SG, Rothmann SA & Robbins WA. (2000) Evaluation of a container for collection and shipment of semen with potential uses in population-based, clinical, and occupational settings. J Androl 21, 478–484. [PubMed: 10819457]

Schisterman EF, Vexler A, Whitcomb BW & Liu A. (2006) The limitations due to exposure detection limits for regression models. American journal of epidemiology 163, 374–383. [PubMed: 16394206]

Schrader SM, Turner TW & Simon SD. (1990) Longitudinal study of semen quality of unexposed workers: sperm head morphometry. J Androl 11, 32–39. [PubMed: 2312397]

Smarr M, Grantz K, Sundaram R, Maisog J, Honda M, Kannan K & Buck Louis G. (2016) Urinary paracetamol and time-to-pregnancy. Human reproduction 31, 2119–2127. [PubMed: 27412248]

Snijder CA, Kortenkamp A, Steegers EA, Jaddoe VW, Hofman A, Hass U & Burdorf A. (2012) Intrauterine exposure to mild analgesics during pregnancy and the occurrence of cryptorchidism and hypospadia in the offspring: the Generation R Study. Human reproduction 27, 1191–1201. [PubMed: 22301570]

Stovall DW, Guzick DS, Berga SL, Krasnow JS & Zeleznik AJ. (1994) Sperm recovery and survival: two tests that predict in vitro fertilization outcome. Fertility and sterility 62, 1244–1249. [PubMed: 7957992]

Thiele K, Kessler T, Arck P, Erhardt A & Tiegs G. (2013) Acetaminophen and pregnancy: short- and long-term consequences for mother and child. Journal of reproductive immunology 97, 128–139. [PubMed: 23432879]

Turner T & Schrader S. (2006) Sperm migration assay as measure of recently ejaculated sperm motility in specimens shipped overnight [Abstract]. J Androl 27**(**suppl**):.():**

Zinaman MJ, Uhler ML, Vertuno E, Fisher SG & Clegg ED. (1996) Evaluation of computer-assisted semen analysis (CASA) with IDENT stain to determine sperm concentration. J Androl 17, 288–292. [PubMed: 8792219]

Table 1.

Characteristics of non-azoospermic male partners (n=468).

Characteristic	n (%)
Age categories	
<30	166 (36)
30–40	282 (60)
40+	20 (4)
Age (years), mean ± SD	31.8 ± 4.8
BMI categories	
Under/healthy (BMI < 25)	80 (17)
Overweight (25 <= BMI < 30)	191 (41)
Obese (30 <= BMI <35)	121 (26)
Morbidly Obese (BMI >= 35)	71 (15)
BMI (kg/m^2), mean ± SD	30.0 ± 5.6
Race/ Ethnicity	
Non-Hispanic White	378 (81)
Non-Hispanic Black	20 (4)
Hispanic	38 (8)
Other	30 (6)
Income	
< $50,000	68 (15)
$50,000 - $99,000	226 (49)
at least $100,000	165 (36)
Abstinence time (days), mean ± SD	4.0 ± 4.5
Conditional Parity	

Characteristic	n (%)
No pregnancies	202 (43)
Never fathered a pregnancy	39 (8)
Fathered a pregnancy	225 (48)

Data presented are from study baseline. Missing data are not reflected in the table.

Table 2.

Distribution of urinary paracetamol and *p*-aminophenol

	n	% >LOD	5th	25th	50th	75th	95th
Paracetamol (ng/mL)	414	93	0.23	5.45	16.03	75.18	54959
p-aminophenol (ng/mL)	414	100	128	493	974	1605	4011

Table 3.

Associations between urinary paracetamol and semen parameters [a]

Semen parameter by paracetamol category [b]	Unadjusted	Adjusted [c]
	β (95% CI)	**β (95% CI)**
General characteristics		
Volume (ml)	−0.05 (−0.10, 0.00)	−0.04 (−0.10, 0.02)
1st quartile	Ref.	Ref.
2nd quartile	**−0.21 (−0.36, −0.06)**	**−0.19 (−0.35, −0.03)**
3rd quartile	**−0.16 (−0.31, −0.01)**	−0.12 (−0.27, 0.04)
4th quartile	**−0.16 (−0.31, −0.01)**	−0.14 (−0.30, 0.02)
p-for linear trend	0.08	**0.04**
Sperm Count (* 10^6/ml)	−0.09 (−0.29, 0.11)	−0.03 (−0.24, 0.18)
1st quartile	Ref.	Ref.
2nd quartile	**1.04 (0.48, 1.6)**	**0.87 (0.29, 1.46)**
3rd quartile	−0.03 (−0.59, 0.54)	0.03 (−0.56, 0.61)
4th quartile	0.08 (−0.48, 0.64)	0.10 (−0.50, 0.69)
p-for linear trend	0.36	**0.01**
Motility		
Average path velocity (μm/sec)	**−1.13 (−2.07, −0.19)**	−0.84 (−1.81, 0.13)
1st quartile	Ref.	Ref.
2nd quartile	0.10 (−2.58, 2.77)	0.38 (−2.39, 3.15)
3rd quartile	−1.11 (−3.8, 1.58)	−0.48 (−3.27, 2.3)
4th quartile	−2.04 (−4.72, 0.63)	−1.73 (−4.54, 1.08)
p-for linear trend	0.09	0.59
Straight-line velocity (μm/sec)	**−0.79 (−1.56, −0.01)**	−0.53 (−1.34, 0.28)
1st quartile	Ref.	Ref.
2nd quartile	−0.25 (−2.47, 1.97)	−0.09 (−2.39, 2.21)
3rd quartile	−1.23 (−3.46, 1.01)	−0.75 (−3.07, 1.56)
4th quartile	−1.38 (−3.6, 0.84)	−1.01 (−3.35, 1.32)
p-for linear trend	0.15	1.0
Curvilinear velocity (μm/sec)	**−2.02 (−3.63, −0.4)**	−1.61 (−3.29, 0.07)
1st quartile	Ref.	Ref.
2nd quartile	−0.01 (−4.62, 4.6)	0.46 (−4.31, 5.23)
3rd quartile	−2.43 (−7.07, 2.21)	−1.61 (−6.4, 3.19)
4th quartile	−4.09 (−8.71, 0.53)	−3.9 (−8.74, 0.94)
p-for linear trend	0.05	0.63
Beat cross frequency (Hz)	**−0.76 (−1.31, −0.22)**	**−0.59 (−1.16, −0.03)**
1st quartile	Ref.	Ref.

Semen parameter by paracetamol category [b]	Unadjusted	Adjusted [c]
	β (95% CI)	**β (95% CI)**
2nd quartile	−0.48 (−2.03, 1.06)	−0.19 (−1.79, 1.42)
3rd quartile	**−2.27 (−3.82, −0.71)**	**−2.13 (−3.74, −0.52)**
4th quartile	**−1.72 (−3.26, −0.17)**	−1.53 (−3.16, 0.09)
p-for linear trend	**0.01**	0.65
Straightness (%)	**−1.99 (−3.46, −0.53)**	−1.46 (−2.98, 0.06)
1st quartile	Ref.	Ref.
2nd quartile	−2.27 (−6.45, 1.90)	−1.76 (−6.09, 2.56)
3rd quartile	**−6.12 (−10.32, −1.92)**	**−5.33 (−9.68, −0.99)**
4th quartile	−4.13 (−8.31, 0.05)	−3.38 (−7.77, 1.01)
p-for linear trend	**0.02**	0.30
Linearity (%)	**−1.03 (−2.02, −0.05)**	−0.64 (−1.67, 0.39)
1st quartile	Ref.	Ref.
2nd quartile	−0.90 (−3.71, 1.91)	−0.62 (−3.55, 2.31)
3rd quartile	**−2.99 (−5.82, −0.16)**	−2.35 (−5.29, 0.59)
4th quartile	−1.85 (−4.66, 0.96)	−1.16 (−4.13, 1.81)
p-for linear trend	0.09	0.52
Morphometry		
Megalo head (%)	0.03 (−0.02, 0.08)	−0.02 (−0.09, 0.04)
1st quartile	Ref.	Ref.
2nd quartile	0.07 (−0.07, 0.21)	0.05 (−0.09, 0.19)
3rd quartile	0.08 (−0.07, 0.22)	0.07 (−0.08, 0.22)
4th quartile	**0.16 (0.02, 0.30)**	0.13 (−0.02, 0.28)
p-for linear trend	**0.04**	0.70
Micro head (%)	−0.03 (−0.07, 0.02)	0.03 (−0.02, 0.08)
1st quartile	Ref.	Ref.
2nd quartile	−0.05 (−0.17, 0.08)	−0.04 (−0.18, 0.09)
3rd quartile	**−0.13 (−0.26, 0.00)** [d]	**−0.16 (−0.3, −0.03)**
4th quartile	−0.12 (−0.25, 0.01)	−0.13 (−0.26, 0.01)
p-for linear trend	**0.03**	0.43
Sperm chromatin stability		
% DNA Fragmentation	0.03 (−0.01, 0.07)	**0.05 (0.01, 0.09)**
1st quartile	Ref.	Ref.
2nd quartile	0.05 (−0.07, 0.16)	0.04 (−0.08, 0.16)
3rd quartile	0.05 (−0.06, 0.16)	0.07 (−0.05, 0.19)
4th quartile	0.10 (−0.01, 0.21)	**0.15 (0.03, 0.27)**
p-for linear trend	0.09	0.85

Note: Bolded effect estimates and 95% CIs indicate statistical significance.

[a] Restricted to parameters with a significant association in unadjusted and/or adjusted models.

[b] Effect estimates and 95% CIs are presented for regression models of continuous log-transformed paracetamol concentration, unless specified otherwise. Paracetamol quartiles: 1st (< 5.44 ng/mL, n=103); 2nd (5.45 – 15.96 ng/mL, n=104); 3rd (16.09 – 74.62 ng/mL, n=103) and 4th (≥75.18 ng/mL, n=104).

[c] Models were adjusted for log-transformed creatinine and age (years).

[d] Statistically significant prior to rounding.

Table 4.

Associations between urinary *p*-aminophenol and semen parameters [a]

Semen parameter by *p*-aminophenol category [b]	Unadjusted	Adjusted [c]
	β (95% CI)	**β (95% CI)**
General characteristics		
Total count (* 10^6 /ml concentration x volume)	−0.22 (−0.52, 0.09)	−0.16 (−0.54, 0.23)
1st quartile	Ref.	Ref.
2nd quartile	−0.42 (−1.3, 0.45)	−0.12 (−1.06, 0.81)
3rd quartile	0.05 (−0.82, 0.92)	0.33 (−0.63, 1.29)
4th quartile	**−1.03 (−1.9, −0.16)**	−0.89 (−1.85, 0.07)
p-for linear trend	0.06	0.58
Motility		
Average path velocity (μm/sec)	0.16 (−0.78, 1.10)	0.57 (−0.59, 1.73)
1st quartile	Ref.	Ref.
2nd quartile	2.22 (−0.47, 4.91)	**2.93 (0.09, 5.78)**
3rd quartile	1.81 (−0.88, 4.49)	2.60 (−0.29, 5.49)
4th quartile	0.55 (−2.12, 3.22)	1.01 (−1.9, 3.92)
p-for linear trend	0.77	**0.02**
Straight-line velocity (μm/sec)	−0.05 (−0.83, 0.73)	0.26 (−0.7, 1.22)
1st quartile	Ref.	Ref.
2nd quartile	1.75 (−0.48, 3.98)	**2.39 (0.04, 4.75)**
3rd quartile	1.24 (−0.99, 3.46)	1.91 (−0.49, 4.31)
4th quartile	−0.02 (−2.23, 2.20)	0.27 (−2.14, 2.68)
p-for linear trend	0.87	**0.02**
Linearity (%)	−0.08 (−1.07, 0.90)	0.50 (−0.72, 1.73)
1st quartile	Ref.	Ref.
2nd quartile	2.71 (−0.11, 5.53)	**3.82 (0.83, 6.82)**
3rd quartile	1.82 (−0.99, 4.64)	**3.19 (0.14, 6.24)**
4th quartile	−0.30 (−3.11, 2.50)	0.58 (−2.48, 3.65)
p-for linear trend	0.69	**0.00**
Morphometry		
Sperm head area (μm^2)	**−0.08 (−0.14, −0.02)**	**−0.10 (−0.18, −0.02)**
1st quartile	Ref.	Ref.
2nd quartile	−0.13 (−0.31, 0.06)	−0.13 (−0.33, 0.07)
3rd quartile	−0.08 (−0.27, 0.1)	−0.10 (−0.3, 0.10)
4th quartile	**−0.26 (−0.44, −0.07)**	**−0.22 (−0.43, −0.02)**
p-for linear trend	**0.01**	0.41
Sperm head width (μm)	**−0.01 (−0.03, 0.00)** [d]	**−0.02 (−0.04, −0.01)**

Semen parameter by *p*-aminophenol category [b]	Unadjusted	Adjusted [c]
	β (95% CI)	**β (95% CI)**
1st quartile	Ref.	Ref.
2nd quartile	−0.03 (−0.06, 0.01)	−0.02 (−0.06, 0.02)
3rd quartile	0.00 (−0.03, 0.04)	0.00 (−0.04, 0.04)
4th quartile	**−0.04 (−0.08, −0.01)**	−0.04 (−0.08, 0.00)
p-for linear trend	0.09	0.54
Sperm head perimeter (μm)	**−0.04 (−0.08, 0.00)**[d]	−0.04 (−0.09, 0.00)
1st quartile	Ref.	Ref.
2nd quartile	−0.04 (−0.15, 0.06)	−0.05 (−0.17, 0.06)
3rd quartile	−0.06 (−0.17, 0.05)	−0.06 (−0.18, 0.05)
4th quartile	**−0.13 (−0.23, −0.02)**	−0.10 (−0.22, 0.01)
p-for linear trend	**0.02**	0.51

Note: Bolded effect estimates and 95% CIs indicate statistical significance.

[a] Restricted to parameters with a significant association in unadjusted and/or adjusted models.

[b] Effect estimates and 95% CIs are presented for regression models of continuous log-transformed *p*-aminophenol concentration, unless specified otherwise. *p*-aminophenol quartiles : 1st (< 491 ng/mL, n=103); 2nd (492 – 969 ng/mL, n=104); 3rd (978 – 1596 ng/mL, n=103) and 4th (≥ 1605 ng/mL, n=104).

[c] Models were adjusted for log-transformed creatinine and age (years).

[d] Statistically significant prior to rounding.

Table 5.

Associations between the sum of urinary paracetamol metabolites and semen parameters [a]

Semen parameter by chemical category [b]	Unadjusted	Adjusted [c]
	β (95% CI)	β (95% CI)
General characteristics		
Total count (* 10^6 /ml concentration x volume)	−0.28 (−0.59, 0.02)	−0.18 (−0.51, 0.15)
1st quartile	Ref.	Ref.
2nd quartile	0.21 (−0.66, 1.07)	0.52 (−0.40, 1.45)
3rd quartile	0.32 (−0.55, 1.19)	0.61 (−0.36, 1.57)
4th quartile	**1.07 (−1.94, −0.21)**	−0.67 (−1.63, 0.28)
p-for linear trend	**0.02**	0.16
Motility		
Average path velocity (μm/sec)	−0.67 (−1.61, 0.27)	−0.41 (−1.41, 0.59)
1st quartile	Ref.	Ref.
2nd quartile	2.59 (−0.08, 5.25)	**2.87 (0.07, 5.68)**
3rd quartile	0.18 (−2.49, 2.86)	0.26 (−2.66, 3.19)
4th quartile	−1.36 (−4.02, 1.29)	−0.52 (−3.41, 2.37)
p-for linear trend	0.13	0.34
Amplitude of lateral head (μm)	−0.10 (−0.21, 0.01)	−0.08 (−0.19, 0.04)
1st quartile	Ref.	Ref.
2nd quartile	−0.06 (−0.37, 0.25)	−0.05 (−0.37, 0.28)
3rd quartile	**−0.37 (−0.68, −0.06)**	**−0.43 (−0.77, −0.09)**
4th quartile	−0.21 (−0.52, 0.10)	−0.20 (−0.54, 0.13)
p-for linear trend	0.06	0.08
Linearity (%)	−0.60 (−1.60, 0.39)	−0.21 (−1.27, 0.85)
1st quartile	Ref.	Ref.
2nd quartile	2.26 (−0.54, 5.06)	**3.13 (0.16, 6.09)**
3rd quartile	1.55 (−1.26, 4.37)	2.50 (−0.59, 5.59)
4th quartile	−1.55 (−4.34, 1.25)	0.13 (−2.92, 3.18)
p-for linear trend	0.23	0.87
Percent Motility (%)	**−0.21 (−0.42, −0.01)**	−0.15 (−0.36, 0.07)
1st quartile	Ref.	Ref.
2nd quartile	0.07 (−0.51, 0.65)	0.23 (−0.39, 0.84)
3rd quartile	−0.13 (−0.72, 0.45)	0.06 (−0.58, 0.70)
4th quartile	−0.46 (−1.04, 0.12)	−0.15 (−0.78, 0.48)
p-for linear trend	0.09	0.52
Morphometry		
Other tail abnormalities (%)	−0.01 (−0.06, 0.04)	0.00 (−0.06, 0.06)

Semen parameter by chemical category [b]	**Unadjusted**	**Adjusted** [c]
	β (95% CI)	**β (95% CI)**
1st quartile	Ref.	Ref.
2nd quartile	**−0.20 (−0.36, −0.05)**	**−0.22 (−0.39, −0.05)**
3rd quartile	−0.07 (−0.23, 0.08)	−0.10 (−0.27, 0.07)
4th quartile	−0.07 (−0.23, 0.08)	−0.07 (−0.24, 0.10)
p-for linear trend	0.73	0.84
Morphology		
Taper (%)	−0.01 (−0.07, 0.06)	−0.01 (−0.08, 0.06)
1st quartile	Ref.	Ref.
2nd quartile	−0.17 (−0.35, 0.02)	**−0.20 (−0.4, −0.01)**
3rd quartile	0.01 (−0.18, 0.19)	−0.06 (−0.26, 0.15)
4th quartile	−0.03 (−0.22, 0.16)	−0.06 (−0.26, 0.14)
p-for linear trend	0.79	0.96

Note: Bolded effect estimates and 95% CIs indicate statistical significance.

[a]Restricted to parameters with a significant association in unadjusted and/or adjusted models.

[b]Effect estimates and 95% CIs are presented for regression models of continuous log-transformed sum of urinary metabolites concentration, unless specified otherwise. Summed urinary paracetamol metabolites quartiles : 1st (< 606 ng/mL, n=103); 2nd (606 – 1113 ng/mL, n=104); 3rd (1114 – 2029 ng/mL, n=103) and 4th (≥ 2083 ng/mL, n=104).

[c]Models were adjusted for log-transformed creatinine and age (years).