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ORIGINAL RESEARCH

Association Between Urinary Parabens and Sperm Quality in Nigerian Men: A Case–Control Study

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Background: Parabens, which are chemicals used as preservatives in cosmetic and pharmaceutical products, have been reported to be associated with low sperm quality in animal and human models. Despite the high exposure of men to paraben-containing products in Nigeria, there are no known studies that investigate the association of parabens with sperm quality in the country.

Objective: To determine the association of urinary levels of metabolites of parabens with sperm count and quality.

Design/Setting: A multicenter case–control study among fertile and infertile men in five hospitals in southern Nigeria. A total of 136 men diagnosed with male infertility (cases) were compared with 154 controls with normal fertility. Urinary levels of parabens (ethyl-paraben, methylparaben, propylparaben, and butylparaben) were measured using liquid chromatography mass spectrometry, while semen analysis and hormone assays were carried out using World Health Organization standards and radioimmunoassay, respectively. Data were analyzed with non-parametric statistics and non-parametric linear regression.

Results: The results showed high levels of parabens in both cases and controls. However, there was no statistically significant difference in urinary levels of ethyl-paraben, methylparaben, propylparaben, and butylparaben between cases and controls. In contrast, propylparaben had a decreasing association with total motility in both groups, but the effect was only statistically significant in the case of male infertility. The results of the regression analysis showed that a unit increase in propylparaben significantly decreased total motility in the cases (infertile men). Similarly, a unit increase in propylparaben decreased morphology significantly in the unadjusted model for infertile men. Only serum testosterone showed an insignificant correlation with urinary parabens.

Conclusion: We conclude that urinary parabens are associated with features of poor sperm quality – motility, morphology, and volume. Measures to reduce exposure of men to agents containing parabens in Nigeria may reduce the prevalence of male infertility in the country.

Keywords: male infertility, Nigeria, parabens, LCMS, case–control study, spermatozoa

Introduction

Parabens (methylparaben, ethylparaben, propylparaben, and butylparaben) are chemicals that are commonly used to preserve cosmetic products. They are found in diverse pharmaceutical products, foods, and beverages, but have recently been recognised to have adverse effects on animal and human reproduction. Studies in animals have reported that exposure to parabens may potentiate estrogenic and other hormonal effects, thereby modulating the outcomes of reproduction.[1,2] Several studies have shown that parabens may impair spermatogenesis, and sperm quality in male rats,[3,4] and mice,[5] by damaging various steps in

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spermatogenesis. Similar effects are increasingly being reported in humans. Several studies have reported that parabens may impair the quality of human spermatozoa[6,7] and inflict damage to sperm DNA in humans.[8] These effects have been attributed to the elicitation of oxidative stress,[9] endocrine disrupting effects,[2] and possibly to the disturbance of cellular mitochondria.[10]

However, a study among Japanese men failed to demonstrate a significant difference in levels of urinary parabens in association with sperm parameters in men attending an infertility clinic.[11] The authors attributed this negative result to either the small sample of only 42 infertile men that participated in the study or to a true evidence of no effect of parabens on sperm parameters. The lack of comparison of the results between men reported as infertile with fertile controls also reduced the internal validity and external generalizability of the results of the study. To the best of our knowledge, very few studies currently exist in the literature that investigate the association of parabens with male infertility in the African population. Given the increasing frequency of reports of male infertility in African countries,[12,13] and the high rates of exposure to parabens,[14,15] it is relevant to determine a possible association between parabens and infertility in African men.

Nigeria has a high rate of male infertility,[16,17] and exposure to parabens in the African continent.[14,18] Parabens are mostly obtained and contaminate humans through makeup, moisturizers, hair care products, and shaving creams. Parabens obtained from these products, as well as those from drinks, food products, and pharmaceuticals, enter the blood stream, from where they potentially affect the male reproductive organ. Nigeria, being a country with high use of these products, we hypothesized that exposure to parabens will likely be a factor in the causation of male infertility in the country.

In 2019, we designed a prospective case–control multi-centre clinical study to investigate the association of the high rate of male infertility in Nigeria with possible exposures to harmful environmental toxins, including parabens. We measured urinary levels of parabens and other reported toxins in men attending infertility clinics in four hospitals in Southern Nigeria, and compared the results with controls of men with reported fertility. The results with mycotoxins have previously been reported elsewhere.[19]

The objective of this paper is to report the results with parabens and answer the research question as to whether levels of urinary parabens are different between fertile and infertile Nigerian men. Specifically, we tested the null hypothesis that urinary parabens will not differ between fertile and infertile men, and that parabens will not influence the various quality parameters of spermatozoa. We believe that the results will be useful in designing measures to reduce the incidence and prevalence of male infertility in Nigeria, with implications for other African countries.

Methodology

Study Design and Population

The research was a component of a larger investigation into the potential link between environmental pollutants and male infertility. In this case–control study, males with low sperm counts and low-quality spermatozoa were compared to men with normal sperm counts and quality regarding the amount of parabens in their urine. The details of the design have been published elsewhere.[19]

Sample Size

Epi-Info was used to calculate the sample size. The study includes 136 males between the ages of 18 and 59 who are infertile (cases) and 154 fertile men in the same age range (controls) who were recruited from two private hospitals, one secondary hospital, and two teaching hospitals in southern Nigeria. The study sites and methodology for determining sample size are described in detail in a published article.[19] Table 1 displays the respondents' distribution by site.

Data Collection

Urine was collected midstream in sterile, clean bottles from both the controls and the patients. To get rid of contaminants, the samples were filtered via a 0.45 membrane filter. The collating hospitals promptly tested the semen samples. In contrast, the filtered urine samples were sent to the central coordinating office, where they were kept in a refrigerator at −4°C until they were ready to be analyzed. Research teams, protocols, participant recruitment methods, and data collection procedures have been extensively detailed elsewhere.[19]

Table 1 Participating Hospitals

Hospital Code	Freq.	Percent
Abel Guobadia	18	6.2
Central Hospital	33	11.4
Graceland	43	14.8
(UBTH)	130	44.8
(UNIMEDTH)	66	22.8
Total	290	

Semen Analysis and Hormone Assay

After at least 3 days without sexual activity, semen samples were obtained from both patients and controls by masturbation. The samples were examined in compliance with the WHO's suggested standards: 1) macroscopically for appearance, volume, pH, liquefaction, and viscosity; and 2) microscopically for motility, viability, total count, and morphology. In accordance with the WHO criteria[20], we classified sperm analysis as normal as follows: 1) volume ≥ 1.4 mls; 2) total sperm count ≥ 39 million; 3) motility ≥ 42%; 4) viability ≥54%; and 5) morphology ≥ 4%.

Serum samples were taken from both cases and controls and analyzed for follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), estrogens, prolactin, and testosterone using kits based on standard ELISA techniques. The semen samples were collected in sterile cups and placed in an incubator at 37°C for 30 to 60 minutes to allow liquefaction before analysis. Then, a manual analysis for the macroscopic and microscopic evaluations of the sample followed which included mainly liquefaction, viscosity, appearance, volume, and pH. Microscopic evaluation included the use of microscopes to determine the presence of round cells, white blood cells, and sperm and agglutination. Then, sample preparation for the sample to allow for the chromatographic analysis to determine the presence of drug residues was performed and this involved solvent extraction and preconcentration of the sample.

Parabens Analysis

The biochemical analysis of metabolites of parabens from the urine samples was conducted for 176 respondents in the Department of Biochemistry at the University of South Africa (UNISA) utilizing Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LCMS/MS).

LC-MS/MS Analysis

The quantification of the parabens followed a LCMS/MS analysis using a previously reported method.[21] The analysis was carried out using Aa Dionex Ultimate 3000 UHPLC system (Dionex Softron GmbH, Dornierstr. 4, Germany). The was done with an ultrahigh resolution quadrupole time-of-flight mass spectrometer Impact II from (Bruker Daltonics GmbH Fahrenheitstr. 4, Bremen, Germany) which was coupled to the UHPLC system with an electrospray ionization and operatesd in positive ion mode. The captired data was processed with software Bruker Compass Data Analysis 4.3. OutSeparation was achieved with an Acquity UPLC® BEH, (Waters, Ireland) while a reversed phase C18 analytical column (Acquity UPLC® BEH, Waters, Ireland) was used to separate the analytes. The ultrahigh resolution quadrupole time-of-flight mass spectrometer Impact II from Bruker Daltonics GmbH Fahrenheitstr. 4, Bremen, Germany, was coupled to this UHPLC system. It has electrospray ionization and operates in positive ion mode.

Sample Preparation

The selected analytes were extracted from urine samples using a solid phase extraction (SPE) method with OASIS HLB cartridges (60 mg, 3 mL). For this process, 200 mL of filtered water samples were spiked with 50 µg/L of the target analytes. Before loading the samples, the HLB cartridges were conditioned with 6 mL of methanol and ultrapure water. Subsequently, the spiked urine samples and method blanks were loaded onto the SPE cartridges at a flow rate ranging

from 5 to 10 mL/min and washed with 5 mL of ultrapure water. Afterward, the cartridges were drained of water for 5 min using a vacuum pump, and the analytes were eluted with 6 mL of methanol: acetonitrile (50:50, v/v). The eluents were concentrated in a vacuum oven at 50°C, reconstituted to a final volume of 0.5 mL in methanol, and then filtered through a 0.22 µm syringe filter into a 2 mL amber glass vial for LCMS/MS analysis.

Quality Assurance/Quality Control

All glassware were thoroughly cleaned prior to washing before each analysis. In order to check for contamination during the extraction stage, analysis of procedural blanks and spiked samples was conducted for each batch of extraction to check for contamination in the extraction process. Likewise, analysis of instrument signal drifts and analyte carry-over were corrected by analyzing instrumental blanks was also carried out to check for carry-over of analytes and drift in the instrument response. Quantification of the analytes was achieved using the external standardization method with a calibration curve obtained by analyzing standard solution with concentrations ranging from 0.25 µg/L to 1000 µg/L ($R^2 > 0.99$). The instrument Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined as the sample concentration corresponding to a signal-to-noise ratio of 3, while the Limit of Quantification (LOQ) was determined as the sample concentration corresponding to and 10 times the signal-to-noise ratio, respectively. Recovery was achieved and was calculated using the equation:

$$\%\,\text{Recovery} = \frac{n_{\text{spiked}} - n_{unspiked}}{spike\ concentration} * 100$$

where, n_{spiked} is the amount of concentration obtained from the analysis of the spiked blank; $n_{unspiked}$ is amount of the standard concentration in the real blank (not-spiked). See Table 2 for details.

Data Analysis

Stata 17 for Windows was used for all analyses. For categorical variables, percentages were used to show the distribution of the study population by sociodemographic and behavioral characteristics, and for continuous variables, the median with interquartile range was used. The case and control groups' categorical variables were tested for significant association using the chi-square test and Fisher's exact test in cases where there are cells with a count of less than five. The non-parametric Mann–Whitney *U*-test was utilized for the other continuous variables that were not normally distributed, while the *t*-test was employed for characteristics like weight that were normally distributed. The test of normality was conducted for all the continuous variables using Shapiro–Wilk test.

The parabens (ethylparaben, methylparaben, propylparaben, and butylparaben) and the sperm parameters (count, active motility, total motility, morphology, and volume), and the serum hormones were continuous variables, and none of them were normally distributed. As a result, the distribution of these data was presented using the median with

Table 2 Linear Range, regression Coefficient, Limit of Detection (LOD), Limit of Quantification (LOQ) of Target Parabens

Compound	Linear concentration (µg/L)	R^2	LOD (µg/L)	LOQ (µg/L)	Spiked Conc. (µg/L)	Recovery (%)	RSD (%)
Methylparaben	0.5–500	0.9999	1.89	6.30	50	73.4	1.32
					125	91.5	0.83
Ethylparaben	0.5–500	0.9999	3.16	10.5	50	83.6	0.87
					125	87.5	0.70
Propylparaben	0.5–500	0.9999	3.66	12.2	50	91.1	1.62
					125	91.7	0.94
Butylparaben	0.5–500	0.9999	2.13	7.10	50	92.9	1.22
					125	92.7	0.91

Note: Coefficient of determination, (R^2) Limit of Detection (LOD), Limit of Quantification (LOQ), Relative Standard Deviation (RSD).

interquartile range, and the Mann–Whitney *U*-test to determine whether there was a significant difference between the case and control groups. Using non-parametric linear regression, the effect of the parabens on the sperm parameters was estimated. Adjusted and unadjusted models were estimated. Models that were unadjusted and adjusted were estimated.

A few variables that were thought to be confounders, derived from previous research, such as age, body mass index, type of occupation and serum hormone (testosterone), were controlled in the adjusted regression models because of the small sample size for the parabens. Spearman correlation performed for the serum hormones and the parabens shows only testosterone as significantly correlated with the parabens, methylparaben and propylparaben. The standard errors were estimated using the bootstrap method. With a 95% confidence interval, all analyses were performed at a statistical significance level of 0.05.

Results

Table 3 displays the distribution of the case and control groups according to their sociodemographic and behavioral characteristics. Except for their BMI, there was no statistically significant difference in the study population between the case and control groups for any of the sociodemographic or behavioral factors.

The distribution of the cases and control by the sperm parameters is presented in Table 4. The cases and control differ significantly in the five sperm parameters. The median for all five sperm parameters is higher in the cases than in the control group.

The median level of ethyl-paraben and methylparaben is higher in the control group than in the cases, and the total study population (Table 5). In contrast, the median level for both cases and control is similar for propylparaben and methylparaben. However, none of the differences are statistically significant.

The results of Spearman rank correlation of the parabens and serum hormones are presented in Table 6. A significant positive relationship was found between testosterone and methylparaben, and propylparaben. Increasing levels of testosterone are correlated with increasing levels of the two parabens. However, the strength of the relationship is small and insignificant.

Table 3 Description of the Study Population by Socio-Demographic and Behavioural Characteristics

Variable	Case (Infertile) N= 136	Control (Fertile) N=154	p-value
	Median (inter-quartile range)		
Age (n=290)	40(10)	40(9)	0.4253
Weight (n=288)	81(21.5)	80(22)	0.4177
Height (n=287)	1.72(0.22)	1.75(0.28)	0.8271
Body mass index (BMI) (n=287)	27(6.79)	26(6.65)	0.0216*
Serum hormones			
Testosterone nmols/l	5.86(6.72)	5.39(6.92)	0.3157
Estradiol nmols/l	59.13(57.83	33.28(42.25)	0.0004*
Prolactin nmols/l	8.27(11.83)	7.73(9.87)	0.5500
FSH nmols/l	7.07(7.82)	5.06(4.6)	0.0006*
LH nmols/l	12.76(8.47)	8.01(9.36)	<0.001*
TSH nmols/l	1.68(2.27)	1.48(1.70)	0.1187

(*Continued*)

Table 3 (Continued).

Variable	Case (Infertile) N= 136	Control (Fertile) N=154	p-value
	Frequency (percentage)		
Marital status(n=277)			
Single	11(8.33)	13(8.97)	
Married	121(91.67)	132(91.03)	0.852
Religion			
Muslim	14(7.79)	12(7.79)	
Christian	118(86.76)	138(89.61)	
Traditional/other	4(2.94)	4(2.60)	0.760
Education			
Primary	7(5.15)	7(4.55)	
Secondary	39(28.68)	35(22.73)	
Tertiary	86(63.24)	107(69.48)	
Other	4(2.94)	5(3.25)	0.676
Occupation			
Agriculture	5(3.68)	5(3.25)	
Business	38(27.94)	47(30.52)	
Skilled manual	15(11.03)	19(12.34)	
Blue collar	19(13.97)	22(14.29)	
Professional	28(20.59)	24(15.58)	
Civil servant	13(9.56)	24(15.58)	
Others	18(13.24)	13(8.44)	0.553
Frequency of alcohol intake (n=281)			
Always	8(6.06)	4(2.68)	
Often	13(9.85)	14(9.40)	
Occasionally	61(46.21)	73(48.99)	
Do not take	50(37.88)	58(38.93)	0.585
Frequency of smoking cigarette (n=268)			
Often	6(4.69)	1(0.71)	
Occasionally	4(3.13)	7(5.00)	
Do not take	118(92.19)	132(94.29)	0.097

Note: *p-value<0.05.

Table 4 Descriptive Analysis of Sperm Parameters (Case, Control, and Total Population)

Fertility Status	Sperm Count	Active Motility	Total Motility	Morphology	Volume
Case (median)	7	10	20	20	2
IQR	16.8	24	31	38	1
Min	0	0	0	0	0.5
Max	76	100	309	80	7.5

(*Continued*)

Table 4 (Continued).

Fertility Status	Sperm Count	Active Motility	Total Motility	Morphology	Volume
Control (median)	46	40	60	57	3
IQR	26	18	17	18	1.5
Min	4.6	9	27	17	0.7
Max	143	85	95	82	7.5
Total (median)	28	30	50	48	2.5
IQR	40	29	38	40	1.2
Min	0	0	0	0	0.5
Max	143	100	309	82	7.5
p-value	<0.001	<0.001	<0.001	<0.001	0.0060

Notes: p-value is from Mann Whitney test; IQR – Inter Quartile Range.

Table 5 Distribution of the Parabens by Fertility Status

Fertility Status	Ethylparaben (N=176) Median(IQR) [Range]	Methylparaben (N=176) Median(IQR) [Range]	Propylparaben (N=176) Median(IQR) [Range]	Butylparaben (N=175) Median(IQR) [Range
Cases (n=86)	0(33) [0–437]	93(353) [0–2020]	0(16) [0–2483]	0(0) [0–55]
Controls (n=90)	4.5(36) [0–941]	111.5(411) [0–1419]	0(19) [0–911]	0(0) [0–55]
Total	3(35) [0–941]	104.5(373.67) [0–2020]	0(18.5) [0–2483]	0(0) [0–55]
p-value	0.4442	0.8740	0.8628	0.2481

Notes: IQR – Inter Quartile Range. The p-value is from the Mann–Whitney *U*-test.

Further investigation using a non-parametric regression model was conducted to examine the effect of the parabens on individual sperm parameters. The results are presented in Table 7. A statistically significant effect of methylparaben on sperm count was observed for men in the control group. An increase in the level of methylparaben increased sperm

Table 6 Spearman Rank Correlation Between the Parabens and Serum Hormones

Serum Hormone	Parabens			
	Ethylparaben	Methylparaben	Propylparaben	Butylparaben
Testosterone				
Rho	0.0596	0.1896*	0.2089*	0.0878
p-value	0.4319	0.0117	0.0054	0.2478
Estradiol				
Rho	−0.1216	−0.1415	−0.1005	−0.0628
p-value	0.1079	0.0610	0.1845	0.4089

(*Continued*)

Table 6 (Continued).

Serum Hormone	Parabens			
	Ethylparaben	**Methylparaben**	**Propylparaben**	**Butylparaben**
Prolactin				
Rho	−0.0816	−0.0272	0.0277	−0.0513
p-value	0.2814	0.7206	0.7148	0.4998
FSH				
Rho	0.0180	−0.0123	0.0750	0.0520
p-value	0.8126	0.8712	0.3226	0.4941
LH				
Rho	−0.0582	−0.0373	−0.0211	0.0148
p-value	0.4431	0.6228	0.7813	0.8456
TSH				
Rho	0.0338	0.0687	0.0429	0.0365
p-value	0.6560	0.3647	0.5716	0.6314

Notes: *p<0.05; rho - Spearman correlation coefficients.

count, but when the effect of testosterone and other factors was adjusted, this relationship became statistically insignificant.

Active motility decreased with increasing ethylparaben, but it only reached statistical significance in the control group. An increasing level of ethylparaben also decreased total motility in both groups but was statistically significant for the control group. Propylparaben had a decreasing effect on total motility in both groups, but the effect was statistically significant for the cases only. A unit increase in propylparaben significantly decreased total motility in the cases by 0.431 holding testosterone and other factors constant.

Table 7 Non-Parametric Regression Analysis Estimating the Marginal Effect of the Parabens on Sperm Parameters

Metabolites	Case		Control	
	Unadjusted Observed Estimate (SE) [95% CI]	**Adjusted Observed Estimate (SE) [95% CI]**	**Unadjusted Observed Estimate (SE) [95% CI]**	**Adjusted Observed Estimate (SE) [95% CI]**
Sperm count				
Ethylparaben	−0.021(0.115) [−0.333–0.118]	−0.107(0.113) [−0.318–0.125]	0.014(0.061) [−0.106–0.133]	−0.010(0.095) [−0.196–0.177]]
Methylparaben	−0.005(0.012) [−0.030–0.019]	−0.002(0.007) [−0.017–0.012]	0.016(0.008)* [0.002–0.031]	0.020(0.029) [−0.035–0.076]
Propylparaben	−0.001(0.028) [−0.056–0.054]	−0.143(0.087)+ [−0.314–0.027]	0.126(0.095) [−0.061–0.313]	0.567(0.439) [−0.293–1.428]
Butylparaben	−0.657(1.336) [−3.275–1.961]	−1.394(1.869) [−5.058–2.26]	0.101(7.612) [−14.819–15.021]	−0.246(3.961)a [−8.009–7.518]

(*Continued*)

Table 7 (Continued).

Metabolites	Case		Control	
	Unadjusted Observed Estimate (SE) [95% CI]	**Adjusted Observed Estimate (SE) [95% CI]**	**Unadjusted Observed Estimate (SE) [95% CI]**	**Adjusted Observed Estimate (SE) [95% CI]**
Active motility				
Ethylparaben	−0.001(0.075) [−0.148–0.147]	−0.028(0.091) [−0.207–0.151]	−0.068(0.022)** [−0.111- −0.025]	−0.115(0.039)** [−0.192- −0.039]
Methylparaben	0.015(0.011) [−0.007–0.037]	0.012(0.021) [−0.030–0.053]	0.008(0.011) [−0.013–0.030]	0.004(0.046) [−0.085–0.093]
Propylparaben	0.056(0.046) [−0.035–0.147]	0.025(0.108) [−0.187–0.237]	−0.010(0.053) [−0.115–0.094]	0.292(0.157) [−0.016–0.600]
Butylparaben	0.268(4.742) [−9.026–9.562]	−1.345(5.151) [−11.439–8.750]	−0.043(2.919) [−5.764–5.678]	−0.099(1.477)[a] [−2.994–2.796]
Total motility				
Ethylparaben	−0.114(0.148) [−0.405–0.176]	−0.250(0.151) [−0.546–0.046]+	−0.050(0.024)* [−0.098- −0.002]	−0.085(0.041)* [−0.165- −0.005]
Methylparaben	0.002(0.026) [−0.048–0.053]	0.021(0.024) [−0.069–0.026]	0.005(0.009) [−0.012–0.022]	0.000(0.032) [−0.062–0.062]
Propylparaben	−0.009(0.042) [−0.091–0.072]	−0.431(0.162)** [−0.749- −0.113]	−0.017(0.042) [−0.098–0.065]	0.003(0.112) [−0.216–0.223]
Butylparaben	1.081(6.532) [−11.722–13.885]	0.435(5.943) [−11.214–12.084]	−0.022(2.948) [−5.801–5.756]	−0.067(1.598)[a] [−3.200–3.065]
Morphology				
Ethylparaben	0.051(0.133) [−0.209–0.311]	−0.083(0.178) [−0.432–0.267]	−0.070(0.057) [−0.183–0.042]	−0.037(0.045) [−0.126–0.052]
Methylparaben	0.042(0.020)* [0.002–0.082]	0.009(0.027) [−0.061–0.044]	−0.008(0.005) [−0.019–0.002]	−0.004(0.042) [−0.086–0.078]
Propylparaben	−0.048(0.021)* [−0.090- −0.006]	−0.037(0.067) [−0.168–0.095]	−0.026(0.045) [−0.114–0.061]	−0.128(0.231) [−0.580–0.324]
Butylparaben	0.280(4.332) [−8.211–8.770]	−0.262(6.577) [−14.152–11.629]	−0.034(3.636) [−7.161–7.092]	−0.112(2.240)[a] [−4.503–4.279]
Volume	I			
Ethylparaben	0.004(0.005) [−0.007–0.015]	0.007(0.010) [−0.012–0.026]	−0.002(0.003) [−0.007–0.004]	0.001(0.003) [−0.006–0.007]
Methylparaben	0.000(0.001) [−0.001–0.002]	0.000(0.002) [−0.004–0.003]	−0.000(0.000) [−0.001–0.000]	0.001(0.002) [−0.002–0.005]
Propylparaben	0.001(0.003) [−0.005–0.007]	0.005(0.006) [−0.008–0.018]	0.001(0.005) [−0.010–0.011]	0.012(0.023) [−0.032–0.057]
Butylparaben	−0.024(0.119) [−0.257–0.208]	0.195(0.618) [−1.015–1.406]	0.023(0.367) [−0.700–0.746]	−0.001(0.040)[a] [−0.080–0.077]

Notes: [a]Only age was controlled due to insufficient observations.
Effect estimates are averages of derivatives. SE is Bootstrap standard error was used because of the small sample size. *p<0.05; **p<0.01.

Sperm morphology increased with increasing levels of methylparaben in the cases but had an insignificant inverse effect in the control group. However, the increasing effect among the cases was only statistically significant in the unadjusted model. A unit increase in propylparaben decreased morphology significantly by 0.048 in the unadjusted model for infertile men (case), but the inverse relationship was insignificant in the adjusted model and in the models for the control group.

Discussion

The objective of the study was to determine whether the urinary levels of metabolites of parabens differed between cases of men with reduced sperm count and control men with normal sperm count. The results showed no statistically significant difference in urinary levels of ethyl-paraben, methylparaben, propylparaben, and methylparaben between cases and controls. This suggests that sperm count, which was used as the key outcome variable in this study, had no significant association with urinary parabens. This result is similar to those of Adoamnei et al[22] which showed no association between urinary parabens and semen characteristics in a cohort of fertile and infertile men in Spain. By contrast, a report among women undergoing intrauterine insemination in Boston, USA,[6] showed that increased paternal urinary levels of parabens decreased the odds of achieving a pregnancy. This suggests that while increasing urinary levels of parabens may not influence overall spermatogenesis and sperm count, it may be associated with sperm quality, which is also an important determinant of reproductive capacity in humans.

With respect to sperm motility, a major feature of male fertility, the results of this study showed that an increasing level of ethyl paraben decreased total motility in both cases and controls but was statistically significant in the unadjusted model for the control group only. In contrast, propylparaben had a decreasing effect on total motility in both groups, but the effect was only statistically significant in cases group. Overall, the results showed that a unit increase in propylparaben significantly decreased total motility in the cases by 0.419 while holding other independent variables constant. Several studies from other parts of the world have similarly showed strong associations between urinary levels of different metabolites of parabens and decreased sperm motility.[23–25]

We also investigated the association between urinary parabens and sperm morphology and volume in fertile and infertile men. The results showed that a unit increase in propylparaben decreased morphology significantly by 0.048 in the unadjusted model for infertile men (case), but the inverse relationship was insignificant in the adjusted model and in the models for the control group. Increased levels of propylparaben had a significantly decreasing effect on volume in the adjusted model for the control group. A one-unit increase in urinary propylparaben decreased the volume by 0.026. This is similar to reports from other studies, which indicate that urinary parabens are correlated with decreasing sperm morphology,[26,27] and volume,[28,29] and may predict male infertility in different populations of men.

Thus, the overall result of this study suggests that increased levels of urinary parabens may be associated with poor sperm quality – motility, morphology, and volume – in Nigerian men. The mechanism responsible for this effect of parabens is not well demonstrated in this study. Although several studies have reported decreased levels of testosterone and sperm quality in men with higher levels of urinary parabens,[24,30] we were able to demonstrate such a relationship in this study.

Study Strengths and Weaknesses

This study has strengths and weaknesses. A major strength is its case–control design utilizing clinical data that focuses on clinically diagnosed cases of male infertility. The use of a comprehensive protocol that investigated these cases prospectively over 3 years ensured accuracy of the data collection methods. The use of the high-fidelity LCMS laboratory method to measure the urinary parabens ensured accuracy of the assay and other data collection methods. Additionally, despite the high exposure of men to products that potentially contain parabens, to our knowledge, this is the first study that investigates the association of parabens with sperm quality in Nigeria. The potential limitation is the fact that urinary samples had to be transferred from Nigeria to South Africa, which may have depreciated the content of the parabens. However, the fact that this potential negative consequence may have occurred equally in both cases and controls diminishes its overall impact on the study results.

Conclusion

We conclude that urinary parabens are not associated with sperm count in Nigerian men, but may be associated with features of poor sperm quality – motility, morphology, and volume. Measures to reduce exposure of men to agents containing parabens in Nigeria may reduce the prevalence of male infertility in the country.

Data Sharing Statement

Data are available on request from the corresponding author.

Ethical Approval and Informed Consent

The research was conducted in compliance with the ethical guidelines set forth by the World Medical Association (Declaration of Helsinki) for studies involving human subjects. Approval was granted by the Ethical Review Committee at the University of Benin. Participants were briefed on the purpose and procedures of the study, and only those who provided consent were enrolled. They were guaranteed confidentiality of their information, ensuring that their identities and contact information would remain undisclosed in the final report of the results.

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Author Contributions

FEO: the conception, design of the study, acquisition of data, interpretation of data, drafting and revision of the article; LFCN: data analysis and interpretation of data; drafting and revision of the article; EU: conception, design of the study, laboratory analysis, acquisition of data, revision of the article critically for important intellectual content; TM: laboratory analysis; revision of the article critically for important intellectual content; CE: acquisition of data, revision of the article critically for important intellectual content; OA: conception, design of the study, acquisition of data, revision of the article critically for important intellectual content; MA: conception, design of the study, acquisition of data, revision of the article critically for important intellectual content; TM: acquisition of data, revision of the article critically for important intellectual content; VIO: acquisition of data, revision of the article critically for important intellectual content; AO: conception, design of the study, acquisition of data, revision of the article critically for important intellectual content; VO: conception, design of the study, acquisition of data, revision of the article critically for important intellectual content; CO: conception, design of the study, acquisition of data, revision of the article critically for important intellectual content; MOA: laboratory analysis, acquisition of data, revision of the article critically for important intellectual content. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

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