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Original Article

Seminal Plasma Bisphenol A Concentrations Among Men with Secondary and Primary Infertility

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Abstract: **Background:** The declining trend of male infertility have been attributed to some factors including environmental, lifestyle behaviors, and dietary habits. Bisphenol A (BPA) which is preponderance in the environment and in various products frequently used has been implicated in male infertility. It is not known whether the seminal fluid BPA concentrations differ among men with primary infertility and secondary infertility. This study was aimed at determining the seminal plasma BPA concentrations among men with secondary infertility and primary infertility and to associated their levels with duration of infertility. **Materials and Methods:** This was a cross-sectional study of 145 men clinically diagnosed with primary infertility (n=80) and secondary infertility (n=65), and men with proven fertility (n=60) as controls. Semen analysis was done manually according World Health Organization guidelines and seminal plasma BPA was assayed using an Elisa kit. Chi square and Student's t-test were used to analyze continuous data and discrete variables respectively. Pearson's correlation coefficient was used to determine the association between seminal plasma BPA and duration of infertility, and a $p<0.05$ was considered statistically significant. **Results:** The seminal plasma BPA was significantly higher ($p<0.001$) among subjects with secondary infertility than those with primary infertility. Sperm count, total motility, progressive motility and normal morphology were lower among secondary infertility than primary infertility, but the mean differences were not significant ($p>0.05$) except viability which was significantly higher among primary infertility than secondary infertility ($p<0.05$). The multiple regression model indicates that, the ages of men (OR=3.26 95% CI 2.23-7.26) was independently associated with BPA concentrations among secondary infertility. The finding of higher BPA concentrations in seminal plasma of men with secondary infertility than primary infertility may be associated with age and not duration of marriage.

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INTRODUCTION

The World Health Organization (WHO), defined Primary infertility as when a pregnancy has never been achieved by an individual while secondary infertility is when a person has had at least one prior pregnancy in the past (WHO, 2013). The declining trend of male infertility have been attributed to some factors such as environmental, lifestyle behaviors, and dietary habits (Babatunde and Emokpae,2022). Male infertility is probably the most neglected and under-emphasized health care issue in our setting and the urgent needs to halt the decline has been emphasized (Uadia and Emokpae, 2015). Fertility care encloses prevention, diagnosis and treatment of infertility (WHO,2023). It is very important to identify those factors that might decrease the chance of fertility among persons with primary or secondary infertility (Benksim et al., 2018).

Bisphenol A (BPA) is a recognized substance that mimic or interfere with the body's hormones. It is present in epoxy resins and polycarbonate plastics which are regularly used in our daily routine. Some authors have shown that BPA exposure has adverse consequences on spermatogenesis via perturbation on the hypothalamic–pituitary–gonadal axis and induction of oxidative stress in testis (Santiago et al., 2021). Studies elsewhere have indicated that because of the preponderance of BPA in the various products frequently used daily, this has occasioned its detection in several human tissues and body fluids including urine, blood, serum, amniotic fluid, and semen (Vandenberg et al., 2007; Vitku et al., 2015). This has raised concerns regarding the possible association of BPA with human disorders (Rochester, 2013; Benksim et al., 2018). Our group has previously reported significantly higher seminal plasma BPA concentrations among men evaluated for infertility in Benin City, Nigeria (Obialor et al., 2023).

It is also important to know whether BPA levels are different between primary and secondary infertility, because serum levels of vitamin E, testosterone, and sperm count were reported to be lower among men with secondary infertility than primary infertility (Emokpae and Moronkeji, 2022). Other authors have observed that male factor infertility is associated with more with primary infertility than secondary infertility (Isong et al., 2017). In secondary infertility, frankly long period of exposure and possible advanced age could increase the chances of exposure and accumulation of BPA in seminal plasma and adverse consequences on sperm quality. This study seeks to determine seminal plasma BPA concentrations among men with secondary infertility and primary infertility and to associated their levels with the ages of infertile men.

MATERIALS AND METHODS

Study design and population

This was a cross-sectional study of men clinically diagnosed with primary infertility (if they have never achieved pregnancy) and secondary infertility (if they had achieved at least one prior pregnancy in the past), and are being investigated for semen analyses. Men with proven fertility as reviewed by the clinician served as controls.

Study site

The specimens were collected from men clinically diagnosed with infertility at the Human Reproduction and Research Programme (HRRP) unit at the University of Benin Teaching Hospital and while control subjects were recruited from fertile men from the same locality.

Inclusion and exclusion criteria
Only subjects who were reviewed and diagnosed with infertility by a clinician and men with proven fertility and normal semen indices (controls) were included in the study. Those males with genital infections and systemic diseases such as hepatic, renal, endocrine, or autoimmune that may impair the reproductive potential and those on thyroid hormone medications or drugs that will affect the results were excluded.

Ethical Consideration
The approval for the study was given by the Health Ethics Review Committee of the University of Benin Teaching Hospital, ref ADM/E22/A/ VOL. VII/14831269 dated February 18, 2022. The study participants were duly informed about the nature of the research in the appropriate language. Also, Informed consent was sought and obtained from every one of them before enrolment. Those who refused to give consent were excluded.

Sample collection
The subjects were tutored on how to collect semen samples by masturbation into wide mouth sterile containers after at least 3 days of sexual abstinence. Caution was taken to ensure that all semen samples were collected into containers without lost. The containers were labeled with time of collection, and number codes and submitted to the laboratory within 1 h of collection.

Sample size calculation
The sample size was calculated using the sample size determination for health studies given as $n = Z^2P(1 - P)/d^2$(Lwanga and Lemeshow,1991) and 10% prevalence of BPA in the seminal fluid of men investigated for infertility (Hanaoka et al., 2002).

Where n = the calculated minimum sample size; z = the standard normal deviation which is usually set at 1.96 and corresponds to a 95% confidence interval; P = the prevalence of BPA in seminal plasma of infertile men from the previous study; q = The proportion in the target population who do not have a particular characteristic, i.e., q = 1-P = 1-0.10 = 0.90; and d = tolerable margin of error, an observed difference of 5% was taken as being significant.
Hence: $n = 1.96^2 \times 0.1 (1 - 0.1)/0.0025$
$=3.8416 \times 0.1 \times 0.9/0.0025$
=138 minimum sample

The sample size used for this study was 145 seminal fluid samples after the addition of 5% attrition. Also, 60 men with proven fertility were enrolled as controls.

Semen analysis
The semen samples were analyzed according to the World Health Organization (WHO) guidelines (WHO, 2010) Semen parameters analyzed are semen volume (mL), sperm motility (%), and abnormal morphology (%) and sperm count. After semen analysis, the specimen was spun in a bucket centrifuge at 2000 rpm for 15 min and the seminal plasma was separated into another clean/sterile container. This was kept frozen at −20°C until BPA analysis was done.

Seminal Plasma BPA Determination
Seminal plasma BPA was assayed using an Elisa kit supplied by MyBioSource Inc., San Diego, CA, USA. The principle of the quantitative sandwich Elisa technique is based on the fact that, when the specimen is added to the BPA Elisa plate precoated with anti-BPA antibody, the antigen–antibody reaction occurs. Thereafter, the wells were washed. The amount of conjugate which was bound to each well was then determined by the amount of color obtained when Tetramethylbenzidine (TMB) was added. The TMB reacted with the Horseradish peroxidase (HRP) available in the well. After the addition of sulfuric acid, the blue-colored product was converted to a yellow-colored product, which was read at 450 nm on a plate reader. The concentration of BPA was extrapolated from a graph previously plotted using various concentrations of standards.

Statistical analysis
Data were analyzed using the Statistical Package for the Social Sciences software (version 20.0, IBM SPSS, Armonk, NY, USA). Chi square was used to analyze continuous data, while Student's t-test was used to compare discrete variables. Pearson's correlation coefficient was used to determine the association between seminal plasma BPA and sperm indices. The level of significance was set at $P < 0.05$.

RESULTS
Table 1 shows the distribution of demographic variables of study participants. It indicates that majority of the respondents were between the ages of 39-46 years, followed by age ranges 47-55 years and 32-38 years. The differences in the age range between infertile group and control group was statistically significant ($p<0.001$)

Table 1: Distribution of Demographic Factors of Respondents

Demographic factors	Infertile group (n=145)	Fertile group (n=60)	X^2	P-value
Age Range (Years)				
32-38	30(61.22%)	19(38.77%)		
39-46	70(76.92%)	21(23.077%)	52.47	0.001
47-55	45(69.23%)	20(30.76%)		
Occupation				
Civil Servant	50(76.92%)	15(23.08%)		
Trader	30(60.0%)	20(40.0%)	9.15	0.027
Drivers	45(81.81%)	10(18.18%)		
Farmers	20(57.14%)	15(42.85%)		
Duration of infertility				
1-5years	86(100.0%)	0(0.00%)	0.53	0.025
6-10years	59(100.0%)	0(0.00%)		

Values in parenthesis are percentage.

Table 2 shows that 80/145 (55.2%) had primary infertility while 65/145 (44.8%) had secondary infertility. The seminal plasma BPA level was significantly higher among subjects with secondary infertility than those with primary infertility. Conversely, Sperm count, total motility, progressive motility and normal morphology were lower among secondary infertility than primary infertility. The mean differences were however not significant except viability which was significantly higher among primary infertility than secondary infertility (p=0.05).

Table 2: Level of Bisphenol A and Seminal Indices among Primary and Secondary Infertility

Parameter	Primary Infertility N=80	Secondary Infertility N=65	P-Value
BPA(ng/mL)	36.82±2.31	51.67±2.95	P=0.001
Total Sperm Count($x10^6$/mL)	8.10±1.18	6.31±1.53	P=0.351
Sperm Count($X10^6$/mL)	3.52±0.54	2.58±0.66	P=0.267
Total Motility(%)	11.05±1.32	7.31±1.72	P=0.082
Prog.Motility(%)	2.26±0.38	1.81±0.47	P=0.459
Viability (%)	26.33±3.13	16.77±3.77	P=0.05
Normal Form(%)	5.47±0.96	4.77±1.22	P=0.646
Volume(mL)	2.51±0.09	2.46±0.88	P=0.671

Value are Expressed in Mean±SD. Prog-Progressive, BPA- Bisphenol A

There was no correlation between seminal plasma BPA concentrations with duration of infertility among primary infertility and secondary infertility. However, using the multiple regression model, the ages of participants (OR=3.26 95% CI 2.23-7.26) and duration of marriage (OR=1.06 95% CI 1.02-2.86) were independently associated with secondary infertility.

Table 3: Level of Seminal plasma BPA based on Duration of Infertility among Primary and Secondary Infertile Subjects

Duration of Infertility					
1 -5 Years			6 -10 Years		
Type of infertility	Primary	Secondary	Primary	Secondary	
Number of Subjects	47	39	33	26	
BPA(ng/mL)	34.7±1.4	43.2±2.9	37.2±3.3	53.8±2.6	
Correlation between BPA and Duration of infertility					
Primary Infertility			Secondary Infertility		
R=0.192 P=0.06	P=0.87		R= 0.238		
Multiple Regression					
Age of men			OR=3.26 95% CI 2.23-7.26		
Duration of Marriage			OR=1.06 95% CI 1.02-2.86		

DISCUSSION

Male factor infertility is a public health challenge in sub-Saharan Africa. However, most of the causes are treatable if correct diagnosis, treatment and therapy are provided. Issues with male or female fertility is often brought to the fore only when the couples start trying to have a conception without success after several attempts. Several factors are adduced to failure to achieve conception ranging from physiological, environmental, and lifestyle behaviours. It was reported that the concentrations of seminal plasma BPA were significantly higher among infertile men than controls (Obialor et al., 2023), but it is not clear whether the burden of BPA in seminal plasma among men with primary infertility is different from those with secondary infertility. This study was aimed at determining whether the increased levels of seminal plasma BPA are different between men with primary infertility and those with secondary infertility in our setting.

In this study the proportion of subjects with primary infertility (55.2%) was higher than those with secondary infertility (44.8%). Primary infertility was the predominant type of male infertility in this study. This does not align with previous study from elsewhere in Nigeria (Emokpae and Moronkeji, 2022), the authors reported rates of primary and secondary male infertility to be 32.3% and 67.7% respectively. The result from the study is lower than 62.9% rate of primary infertility reported in Lagos, Nigeria (Jeje et al., 2016), 59% rate of primary infertility observed in Nnewi, southeast Nigeria (Nwajiaku et al.,2012). The observed rates were lower than rates reported from outside Nigeria (Abdulla, 2011; Al-Turki, 2015;Oztekin et al., 2019). In Saudi Arabia, a rate of 78.9% primary male infertility was reported (Al-Turkin, 2015), while Abdalla (2011) observed a rate of 77.4% primary male infertility in Sudan, and a rate of 77.4% primary male infertility was reported in Turkey (2019).

In this study, the poor semen quality was common to both primary and secondary infertile men. Specifically, the difference in the mean sperm count was not statistically significant when compared between primary and secondary infertility. This finding is not consistent with previous study (Emokpae and Moronkeji, 2022), where the authors reported significantly lower ($p<0.05$) sperm count among secondary infertile men than primary infertile men in Osogbo, southwest Nigeria.

The concentration of seminal plasma BPA among men with secondary infertility was significantly higher ($p<0.001$) than in men with primary infertility. The impact of the higher BPA levels in secondary infertile men was evidenced by its impact on sperm viability which was significantly lower among secondary infertile men compared with primary infertile men. To the best of our knowledge, there is paucity of information regarding the levels of BPA in seminal plasma of men with primary and secondary infertility. The reason(s) for the differences in the concentrations is not understood, but the multiple regression analysis revealed that age of men was associated with BPA concentration among secondary infertile men. In secondary infertile men, advanced age may be responsible for the higher concentration of BPA in seminal plasma than primary infertile men. There was no association between seminal plasma BPA and duration of infertility. The commonest pathway of BPA entry into the human is through food and drinks stored in containers made of plastics containing BPA in a polymerized form. Exposure of such vessels containing food and drinks to high temperatures release the BPA monomers into the content and can be ingested into the body (Jeseta et al., 2022). Other routes of exposure include inhalation and body contact.

Once ingested via the gut, BPA is readily absorbed into the circulation across the intestine and is metabolized by the liver. In the liver, BPA is detoxified by conjugation using uridine diphosphate glucuronyltransferase or sulfotransferases to remove the estrogenic activity of BPA (Presunto et al., 2023). However, about 99% free or unconjugated BPA which is regarded as the active harmful form with endocrine disrupting properties are eliminated in feces and urine. Only 1% is believed to accumulate in tissues. Interestingly, BPA entering in the body through dermal contact may not be metabolized in the liver, but can result in excessive bioaccumulation of free BPA in the blood (Presunto et al., 2023).

The estrogen and anti-androgen effects of BPA can damage the development and function of Leydig cells and may cause related reproductive diseases including testicular dysgenesis syndrome, subfertility and infertility (Li et al., 2020). The endocrine disruption effects of BPA is due to its structural similarity to 17 beta-estradiol. BPA can mimic the actions of estrogen by tethering to nuclear estrogen receptors to initiate estrogenic actions. It can also tether to G-protein-coupled estrogen, gamma peroxisome proliferator-activated, and orphan nuclear estrogen gamma receptors, to disrupt cellular and endocrine actions (Jeseta et al., 2022; Presunto et al., 2023). BPA is capable of competing with endogenous estradiol for estrogen receptors, thus acting as an estrogen antagonist.

BPA can hamper male reproductive potential by interfering with the hypothalamic-pituitary-testicular axis and changing the molecular expression and activity of enzymes involved with testicular steroidogenesis and spermatogenesis. It was also observed that BPA directly causes sperm damage by repressing the antioxidant status and leading to increased oxidative stress. All these contribute to poor sperm quality and infertility.

Recently, BPA was reported to alter epigenetic regulation and the histone-toprotamine (PRM) transition (Ryu et al., 2022). The exposure of male mice to BPA for 6 weeks resulted in the alteration of the mRNA levels of the histone family and PRMs significantly. The regulation of the PRMs is a foundational process for male fertility in testes and sperm characteristics. The authors reported that, 'core histone proteins, the PRM1/PRM2 ratio, directly linked to male fertility, and transition proteins were significantly reduced'. Abnormal histone-to-protamine substitution during spermiogenesis also occurred. Subsequently, the amount of histone H3 modification within the testes and DNA methylation in sperm cells were elevated (Ryu et al., 2022).

It was previously advocated that governments should leverage on the recent evidence of deleterious effects of BPA on male infertility by identifying the sources of contamination, safety levels, and harmful effects of BPA and act appropriately (Obialor et al., 2023). Conversely, a report by the Consortium Linking Academic and Regulatory Insights on BPA Toxicity has intensified the controversy regarding the developmental hazards' effects of BPA exposure. Data from that study suggested that there was no evidence of nonmonotonic dose response or significant deleterious effects of developmental exposure later in life among experimental animals (Camacho et al., 2019).

CONCLUSION

The levels of seminal plasma BPA was significantly higher among secondary infertile men than primary infertile men. This difference may be associated with age and not duration of marriage. The possible damaging effects of BPA exposure to male infertility should be of great concern because of the ubiquitous nature of the compound in the environment and foods.

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