RELEVANCE OF SOME PESTICIDES IN SEMEN QUALITY OF INFERTILE MEN IN ADO –EKITI LOCAL GOVERNMENT AREA OF SOUTH WEST NIGERIA

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DOI: 10.5281/zenodo.12580466

Abstract

Background: Infertility and poor semen quality have recently been linked to pesticide exposure due to agricultural practices and environmental contamination. **Objective:** This research was carried out to determine whether pesticides in seminal fluid of men in Ado-Ekiti, South western Nigeria could have a relationship with their fertility status. **Methodology:** Male volunteers whose wives were being investigated for infertility in Ekiti State University Teaching Hospital and some private Clinics in Ado-Ekiti were co opted into the study. Following the completion of the questionaires and signing of the consent forms, Semen samples were obtained from the respondents by masturbation, analyzed and classified into normospermic, asthenospermic, oligospermic and azospermic groups using WHO criteria. Seminal plasmas were obtained by centrifugation and stored frozen. Twenty samples in each of the groups were estimated for their pesticides contents using High Performance Liquid Chromatography and the results obtained analyzed using SPSS Version 24. **Results:** The results showed that the concentrations of dichlorodiphenyltrichloroethane (DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDE), methoxyechlor, aldrin, heptachlor, chlordae, endosulfan, mirex, toxaphene, eldrin, dichlofor, pertane, thiodone, malathion, parathion, phorate, dichlorvos, cyclodiegenes, lindane, cyclohezane, hexachlorobenzine, pentachlorobenzene, and menzon were all significantly higher at $P<0.05$ in all the abnormal groups compared with the Normospermic group. **Conclusion:** Pesticides are associated with sperm abnormalities and infertility. It is recommended that routine semen assessment for pesticides be included in the diagnosis of male infertility and legislation enacted regarding the use of pesticides.

Keywords: Pesticides, Male infertility, Normospermia, Asthenospermia, Oligospermia, Azoospermia.

INTRODUCTION

Infertility a significant global issue, affects 15% of all couples who are of reproductive age. Male variables, such as lower-quality semen, account for 25% of these cases. Currently, the etiology of sub optimal semen quality is poorly understood, and many physiological, environmental, nutritional and genetic factors, have been implicated (Mumtaz *et al*.,2013). Several studies have shown that the decline in sperm parameters could be linked to environmental toxins and pollutants (Mima *et al*.,2018, Mann *et al* 2020). The observed global decline in semen quality in recent times have been associated with the upsurge of endocrine disrupting chemicals present in many pesticides (Knapke *et al.,* 2022). Between 1991 and the present day, many studies and reviews have established a close relationship between environmental exposure to pesticides and declining semen quality (Knapke *et al.,* 2022). Pesticides are used widely in production and preservation of agricultural products. The 20th century saw an expansion in world population, which was made feasible by improvements in food supply and preservation of which the use of pesticides play a key role (Tudi *et al.,* 2021). Aside from agricultural uses, these substances are also used in the urban areas for the maintenance of buildings, warehouses and green zones (Nicolopoulou-Stamati *et al.,* 2016). Pesticides contain a mixture of several chemicals such as organophosphates, carbamates, organochlorines, triazines, synthetic pyrethroids and neonicotinoids. These chemicals have been linked to several issues of public health concerns (Martenies and Perry, 2013). Organochlorines such as DDT and its metabolite DDE have been shown to possess endocrine disrupting properties affecting the pituitary gonadal axis (Martenies and Perry, 2013; Turusov *et al.,*2002). Spermatogenesis is regulated by the pituitary –testicular axis of the male endocrine system and as such pesticides and their metabolites that mimic the natural male hormones can adversely affect semen quality and quantity(Dohle *et al.,*2003). The environmental contamination by the organochlorine DDT has also been associated with decreased spermatozoa motility, concentration and vitality in infertile men (Giulioni *et al.,* 2022). Pesticides have also been shown to possess genotoxic properties, causing epigenetic changes, DNA damage and consequent disruption of the normal metabolism in the pituitary testicular axis (Lopes-Ferreira *et al.,* 2022). Because pesticide are used to destroy pests and other insects, they also exhibit toxicity to other useful (beneficial) organisms such as birds, fish , plants and their applications equally pollutes the air, water, soil and crops (Tudi *et al.,* 2021). It has also been established that exposure to pesticides causes health issues such as ovarian and lung cancers, genetic damages, sperm DNA damage and Parkinson disease (Alengebawy *et al.,* 2021).

Ekiti State is an agrarian zone and the use of pesticides is rampant both in the urban and rural population. Male infertility is a major health challenge in the area and is one of the key causes of marital stress. The association between semen quality and insecticides which has been documented in many countries has not been evaluated in Ado-Ekiti hence this research work was carried out.

RESEARCH METHODS

Study setting and design: A cross-sectional study was conducted in Ekiti State University Teaching Hospital (EKSUTH) and Echoes of Grace Medical Diagnostics and Research Laboratories both in Ado-Ekiti, South Western Nigeria between August November 2022 and June, 2023 among men who were being evaluated for infertility. Semen samples were obtained from the participants by masturbation after abstaining from sexual intercourse for 4-6 days. Semen analysis were performed and the specimens categorised into normospermic, asthenospermic, oligospermic and azoospemic based on the WHO criteria (WHO 2021). The semen samples were centrifuged hard at 3000rpm for 5 minutes and the supernatant plasmas separated, labeled and frozen until biochemical analysis was carried out.

Study population: Twenty samples each of the normospermic, asthenospermic, oligospermic and azoospermic groups were selected out for further investigations on their pesticide content.

Biochemical analysis: The seminal plasma samples were subjected to acetonitrile extraction/partitioning and dispersive solid-phase extraction process after which they were injected into the HPLC system where they were separated based on the affinity of the various pesticides for the stationary phase and the mobile phase. The separated compounds were then detected and quantitated using UV-Visible spectroscopy and appropriate standards (Anastassiades *et al.,*2003).

Statistical analysis: Statistical analysis was performed using the SPSS Version 25 (IBM® SPSS® Statistics; Chicago, IL, USA 2016). The means and standard variations of the various pesticides were determined and T test was used to compare the normospermic group separately with the asthenospermic, oligospermic and azoospermic groups at corresponding 95% confidence interval. For all tests, statistical significance was set at a p-value < 0.05.

Ethical considerations: Ethical approval was obtained from the Research and Ethics Committee of Ekiti State University Teaching Hospital, Ado-Ekiti with the reference number-EKSUTH/A67/2018/08/004. Each of the participants signed the consent forms before the commencement of the study.

RESULTS

Table 1 shows the concentrations of organochlorines in the semen of the four classes of semen samples. The concentrations of the pesticides in the asthenospermic, Oligospermic and azospermic subjects were significantly higher than those of the normospermic at P<0.05. No significant differences are noticed between the concentrations of the pesticides in the asthenospermic and oligospermic groups, however the concentrations of the pesticides in the azospermic group is significantly higher than those of asthenospermic and oligospermic groups.

Table 1: Concentrations of Organochlorine Pesticides in the seminal plasma of fertile and infertile men

Pesticides concentration in µg/l	Normospermia	Asthenospermia	Oligospermia	Azospermia
DDT	6.29±2.60[a]	18.74±8.68 [b]	17.04±9.42[b]	27.19±14.51[c]
DDD	1.23±0.58[a]	3.66±1.70[b]	3.33±1.84[b]	5.31±2.83[c]
Methoxyehlor	0.94±0.47[a]	2.81±1.30[b]	2.55±1.41[b]	4.67±2.17[c]
Aldrin	3.93±1.86[a]	11.70±5.42[b]	10.64±5.88[b]	16.97±9.06[c]
Dieldrin	1.22±0.58[a]	3.63±1.68[b]	3.30±1.83[b]	6.53±2.81[c]
Heptachlor	1.61±0.76[a]	4.79±2.22[b]	4.35±2.41[b]	6.94±3.70[c]
Chlordane	16.07±7.60[a]	47.89±22.17[b]	43.54±24.07[b]	69.46±37.06[c]
Endosulfan	6.80±3.22[a]	20.25±9.38[b]	18.41±10.18[b]	29.38±15.67[c]
Mirex× 10^{-3}	3.80±1.80[a]	11.32±5.24[b]	10.29±5.69[b]	16.43±8.76[c]
Toxaphene	5.47±2.59[a]	16.31±7.55[b]	14.82±8.20[b]	23.65±12.62[c]
Eldrin 10^{-3}	5.14±2.43[a]	15.32±7.09[b]	13.93±7.70[b]	22.22±11.85[c]
Dicofol 10^{-3}	9.44±4.47[a]	28.14±13.03[b]	25.58±14.14[b]	40.81±21.77[c]
Pertane 10^{-3}	35.39±16.74[a]	105.43±48.82[b]	95.84±52.99[b]	152.93±18.16[c]
Thiodon 10^{-3}	5.82±2.75[a]	17.34±8.03[b]	15.76±8.71[b]	25.15±13.42[c]

Key: Values are means of triplicate ± SD. Samples carrying the same superscripts in the same row are not significantly different at [p<0.05]

Table 2 shows the concentrations of organophosphates in the semen of the four classes of semen samples. The concentrations of the pesticides in the asthenospermic, oligospermic and azospermic subjects were significantly higher than those of the normospermic at P<0.05. There were no significant differences between the concentrations of malathion, parathion and dichlovos in the asthenospermic and oligospermic groups and they are significantly lower compared with the azospermic group. The concentrations of phorate does not differ significantly between asthenospermic and azospermic groups whereas phorate concentration in the oligospermic group is significantly higher than those of asthenospermic and azospermic groups.

Table 2: Concentrations of Organophosphate Pesticides in the seminal plasma of fertile and infertile men

Pesticides concentration in µg/l	Normospermia	Asthenospermia	Oligospermia	Azospermia
Malathion	1.20±0.57[a]	3.59±1.66[b]	3.26±1.80[b]	5.20±2.77[c]
Parathion	11.84±5.60[a]	35.27±16.33[b]	32.07±17.73[b]	51.16±27.30[c]
Phorate	1.12±0.53[a]	3.33±1.54[b]	12.27±6.78[c]	4.83±2.58[b]
Dichlorvos	8.77±4.15[a]	26.13±12.10[b]	31.8±1.76[b]	37.90±20.22[c]

Key:Values are means of triplicate ± SD.

Samples carrying the same superscripts in the same row are not significantly different at [p<0.05]

Table 3 shows the concentrations of benzene based pesticides in the semen of the four classes of semen samples.The concentrations of the pesticides in the asthenospermic, oligospermic and azospermic subjects are significantly higher than those of the normospermic at P<0.05.

Table 3: Concentrations of benzene based pesticides in the seminal plasma of fertile and infertile men

Pesticides concentration in µg/l	Normospermia	Asthenospermia	Oligospermia	Azospermia
Cyclodienges	5.94±2.81[a]	17.68±8.19[b]	16.08±8.89[b]	25.65±13.69[c]
Lindane	1.16±0.56[a]	18.30±0.85[b]	16.64±0.92[b]	26.60±1.42[c]
Cyclohexane	1.89±0.89[a]	5.62±2.60[b]	5.11±2.82[b]	8.15±4.39[c]
Hexachlorobenzine	4.91±2.32[a]	14.63±6.78[b]	13.30±7.35[b]	21.22±11.32[c]
Pentachlorobenzine	1.38±0.65[a]	4.12±1.90[b]	3.75±2.07[b]	5.80±3.19[c]
Menzon	3.42±1.62[a]	10.18±4.71[b]	9.25±5.12[b]	14.76±7.88[c]

Key:Values are means of triplicate ± SD.

Samples carrying the same superscripts in the same row are not significantly different at [p<0.05]

Figure 1 shows the illustration (histogram presentations) of the overall concentrations in µg/l of the total pesticides in the seminal plasma of the respondents. The total detectable pesticides in the azospermic group are the highest while that of the normospermic group is the least. That of the asthenospermic group is slightly higher than the oligospermic group.

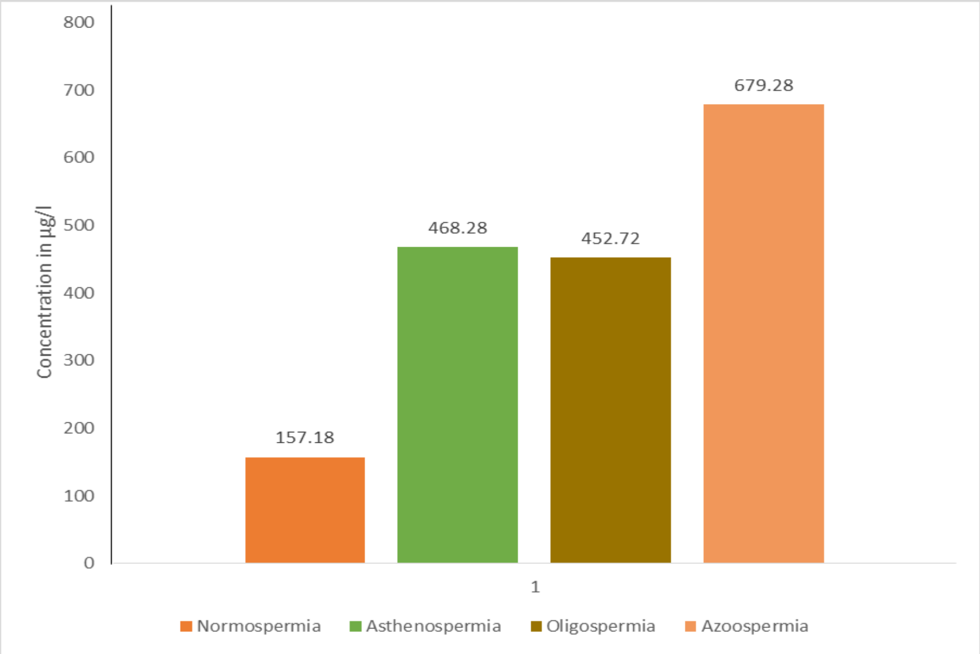

Figure 1: Total pesticides in the seminal plasma

DISCUSSION

This study evaluates the concentrations of environmental pollutants pesticides in semen of various classes of infertile men compared with the normal. Our findings in this study shows that seminal plasma concentrations of various pesticides are significantly increased in asthenospermic, oligospermic and azoospermic subjects compared with the normospermic subjects at $P<0.05$ indicating that pesticides in semen are associated with decline semen quality and quantity. This supports the reports of Sengupta and Banerjee, 2014. Pesticides are divided into three groups in this study namely the organochlorines, organophosphates and benzene based pesticides.

Our study indicates that the levels of all the organochlorine pesticides are significantly increased in asthenospermia, oligospermia and Azoospermia compared with the normal group. These observations supports the findings of Giulioni *et al.,*2022 and Lopes-Ferreira *et al.,* 2022. The mechanism behind these findings are that organochlorine pesticides such as DDT have been identified as endocrine disruptors which act directly on reproductive systems by blocking the actions of androgen receptors. Spermatogenesis is controlled in the body by hormones including testosterone which is the major androgen (Sengupta P, and Banerjee, 2014, Martenies and Perry, 2013). These synthetic polychlorinated biphenyls contain phenolic moiety that mimics the natural hormones. While the natural hormones act in low concentrations, are dose dependent and responds to the normal feed back mechanisms, these endocrine disruptors do not obey the normal metabolic dose-response effects and lower concentrations may cause stronger effects than higher doses (Gray, 1998). The findings in this study corroborates previous findings of Haugen *et al.,* 2011 and Messaros *et al.,* 2009). The association of increased organochlorines with oligospermia and azoospermia as seen in this study has also been established in previous studies of Aneck-Habn *et al.,* 2007. Increased organochlorines has also been associated with asthenospermia, oligospermia and teratospermia (Pant *et al.,*2004; Martenies and Perry 2013). Pertane an isoprocarb is a carbamate of the organochlorine family widely used in local market. The present study shows that perthane toxicity affects male fertility leading to reduced sperm motility (asthenospermia) oligospermia and azoospermia. Its role as a strong endocrine disruptor of the male reproductive system has been reported in previous studies of Zhou and Fang, 2015.

Organophosphates are also pesticides which disrupts the endocrine system (Lopes-Ferreira *et al.,* 2022;Perry *et al.,* 2011, Hernández *et al.,* 2013). In this study, concentrations of organophosphates increased progressively with asthenospermia, oligospermia and the highest concentrations are found in azoospermic subjects. These findings support the reports of Hernandez et al., 2013. Organophosphates exerts their actions at the molecular level causing increasing estrogenic effects, manifesting as feminization which adversely affects spermatogenesis (De Silva *et al.,*2006). Organophosphates has also been associated with increased oxidative stress , synthesis of free radicals and lipid generation all of which cummulate in defects of spermatogenesis manifesting as asthenospermia, oligospermia and azoospermia (De Silva *et al.,*2006). Dichlorvos, one of the organophosphates analysed in this study is a common insecticide used domestically in controlling insects. This study showed that dichlorvos concentrations are increased in asthenospermia , oligospermia and azospermia compared with the normospermia group. These findings support the works of Faris, 2008 and Ezeji *et al.,* 2015]. Malathion is an endocrine disruptor with carcinogenic effects. It increases the concentration of acetylcholine in the blood and reduces sperm viability in pigs (Betancourt *et al.,* 2006). It binds acelylcholinesterase and acetycholine both of which are involved in neurotransmission thus disrupting the normal stimulation of the nerve cells (Reigart and Roberts, 1999. Malathion also causes hyperprolactinaemia which in turn diminishes the process of spermatogenesis (Simionescu *et al.,* 1977). These findings are supported ny the previous reports of of Srinivasan *et al.,*,2005.

Benzene and its products(Lindane, kepone and pentachloro benzene insecticides) have been implicated in defects of spermatogenesis manifested by oligospermia, abnormal sperm cells and asthenospermia. Benzene cause destruction of leydig cells Archibong *et al.,* 2008). Lindane , kepone and pentachloro benzene insecticides have been banned by Stockholm convention because of their adverse effects on human health (Madaj *et al.,*2018). They also affect the human reproductive system and reduces sperm cell counts(Amir *et al.,*2021). Lindane exerts toxic effects on the testis with generalized derangement in the entire male reproductive system including testosterone metabolism (Zhang *et al.,* 2019). Chlodane and lindane have been associated with hypothyroidism and this also contributes to infertility in both males and females (Shrestha *et al.,* 2018).

CONCLUSION

In general, our findings in this study support the previous submissions which highlights the adverse effects of pesticides on the male reproductive system. Pesticides interactions interferes with the synthesis and functions of reproductive hormones, cause mitochondrial damage, oxidative stress and general dysfunction of cells and tissues in the reproductive system including the epididymis, Sertoli cells and germinal epithelia (Moreira *et al.,* 2021).

Competing interests

The authors declare no competing interest.

Authors' contributions

DDA is the first author for this study and undertook data collection, analysis, and wrote the first draft. DDA conceptualized the study; DDA and OBA lead and designed the study; DDA and OBA performed logistical planning, allocation, and implementation; DDA and OEA performed literature search; JOA performed data extraction; JOA and OEA performed risk of bias assessment; JOA and SOA performed initial statistics execution, DDA composed the manuscript; OBA and SOA reviewed statistical approach and DDA executed the study; DDA, OEA, and OBA reviewed the draft; and AOB and OEA reviewed and revised the study and approved the final manuscript for submission.

Acknowledgements

The authors acknowledge the support of the Medical Laboratory Scientists of Ekiti State University Teaching Hospital, Ado-Ekiti and the staff of Echoes of Grace Medical Diagnostics and Research Laboratory, Ado-Ekiti during the period of sample collection from the respondents.

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