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Herbicide Residues: Exploring Their Potential Impact on Semen Quality and Spermatogenesis Among Males Attending Infertility Clinics at Ekiti State University Teaching Hospital, Ado-Ekiti, Western Nigeria.

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ABSTRACT

Background: Reports from various countries have consistently demonstrated a correlation between herbicide exposure, arising from agricultural practices, and a decline in semen quality, leading to male infertility. This study was conducted at Ekiti State University Teaching Hospital in Ado-Ekiti, a rural community characterized by a predominantly agrarian population to determine the relationship between semen quality and herbicides residues.

Methods: The study focused on males whose spouses were seeking assistance at infertility clinics. Routine semen analyses were performed according to the World Health Organization (WHO) criteria, categorizing samples into normospermic, asthenospermic, oligospermic, and azoospermic groups. Seminal plasma samples from each group (twenty samples per group) were subjected to analysis for the presence and concentration of herbicides using High-Performance Liquid Chromatography (HPLC). The following herbicides were investigated: halosulfurum, linuron, fluometuron, chlorimuron, imaxamox, cloransulam, dicamba, fluroxypor, trichlopyr, propanil, cloclinafop, clethodim, quizalofop, fluazifop, pinoxaden, bentazon, atrazine, and bromoxynil. The obtained results were subjected to statistical analysis using SPSS version 24.

Results: The analysis revealed significantly higher concentrations of most herbicides in the asthenospermia, oligospermia, and azoospermia groups compared to the normospermic group ($P<0.05$). These findings suggest a strong association between herbicide exposure and poor semen quality in the studied population.

Conclusions: This study provides compelling evidence supporting the hypothesis that herbicides exposure could be a contributory factor to diminished semen quality in the investigated rural community. The results underscore the importance of considering seminal herbicide determination as a routine component in male infertility testing. Additionally, the study advocates for the implementation of relevant legislation to mitigate potential risks associated with herbicide exposure.

Key words: Herbicides, Spermatogenesis, Semen quality, Endocrine disruption, Oxidative stress.

1. INTRODUCTION

Recent reports from various countries have consistently demonstrated an alarming rate of declining semen quality and its relationship with herbicide exposure, due to agricultural practices leading to male infertility[1,2]. This present crisis indicates that in some countries, exposure to some herbicides are responsible for 3-9 times greater risk of having abnormal semen[2]. Infertility is a major cause of emotional and marital distress all over the world. It affects around 8–12% of couples, with male-factors identified as the primary cause in 20-30 % of cases.[3] Environmental pollution has emerged as a major cause for the rising trend of male infertility in today's era all over the world due to the universal presence of environmental contaminants especially through agricultural practices[4,5]. The risk of sperm abnormalities and decreased fertility have been linked to human exposure to certain herbicides commonly used for agricultural purposes[4]. In developing nations, high exposure occurs due to lack or non-availability of protective clothing. Most classes of herbicides such as ureas, sulfonyureas, bipyridyliums, synthetic auxins and branched chain amino acid inhibitors have been shown to

adversely affect spermatogenesis when humans are exposed to sufficient doses either through water, food and environmental inhalation[4].

Herbicides are substances used to control undesired vegetation. They are used mostly on farmland, however, they are also applied in urban areas for the maintenance of green zones, sport fields, recreation centers, and residential areas[6]. Mixtures of different chemicals are used to compose various types of herbicides depending on the purpose of its use. The commonest types are the aromatic amino acid inhibitors such as glyphosate and sulfosate commonly found in roundup, rodeo and touch down; branched chain amino acid inhibitors which includes the imidazolinones (Imazquin, imazethapyr, imazapyr), sulfonylureas, chlorimiruon , chlorsulfuran, nicosulfuran etc). Others are chlorophyll carotene pigment inhibitors, lipid biosynthesis inhibitors (Triazines), cell division inhibitors (Dinitroanilines), shoot inhibitors (thiocarbanates) and root inhibitors such as bensulide, neopropanamide and propamide)[7].

There are different mechanisms by which herbicides affect the male reproductive system culminating in decreased sperm count, abnormal morphology and poor motility. Effective spermatogenesis depends much on the normal rhythmic secretions of all the reproductive hormones. Some herbicides have been associated with disruption of hypothalamic-pituitary-gonadal axis thus affecting semen production[8]. Herbicides that have been implicated in the endocrine disruption includes dicamba, fluroxypyr, atrazine, bromoxynil, linuron and trichlopyr[9,10,11]. Some herbicides such as atrazine, propanil, and linuron actually exhibit anti androgenic properties inhibiting testosterone secretion while promoting the synthesis of oestrogen resulting in feminization in the males, a process which leads to oligospermia, azospermia and decreased libido[11,12].

Some herbicides like halosulfuron, linuron, fluroxypyr, fluazifop and bromoxynil have also been implicated in the generation of free radicals, oxidative stress and inflammation which antagonize the normal physiology of the testis resulting in male infertility [13,14,15,16]. Oxidative stress is the net result of imbalance between the levels of reactive oxygen species and the counter mechanism of antioxidants. Increase concentrations of reactive oxygen species (ROS) has been identified as a causative factor in the development of asthenospermia, oligospermia and male infertility[17,18]. Inflammation on the other hand is a biological response of the body to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective mechanism that involves the immune system's response to eliminate the cause of cell injury and initiate the healing process. Inflammation is associated with asthenospermia, oligospermia and azoospermia. Chronic inflammation in the reproductive tract can lead to structural damage to the testes, epididymis, or other parts of the male reproductive system, affecting sperm production, maturation, and transport[19,20].

Furthermore, some herbicides like linuron, dicamba and clethodim have been shown to cause DNA and testicular damage, leading to oligospermia and azoospermia[21,22]. Mutagenicity in the male reproductive system has been associated with herbicides such as propanil, quizalofop, fluometuron, and linuron[23,24]. The herbicides immaxamox, clodinafop, fluazifop and pixoxaden disrupt lipid synthesis, a necessary process which is required for teststerone production in the body[25], while some herbicides like immaxamox, cloransulam, clethodim and fluazifop are enzyme inhibitors which interfere with many metabolic pathways in the body [15,25,26]. It has been advocated that some herbicides such as glyphosate be banned in agricultural practice while some countries have actually banned it because of its numerous health hazards[27].

Environmental pollution due to herbicides is widespread and has been shown to have adverse effects on human fertility. Investigating their impact on male fertility is essential for understanding the potential risks and designing appropriate preventive measures to safeguard public health. Despite, the adverse impacts of herbicides on spermatogenesis in adult men, there is paucity of available data on the direct impact of these chemicals on men in the study area. The available studies are usually in occupational settings where the people are exposed to these substances at very high concentrations and not for the general population[28]. Agricultural practice is the main occupation in Ekiti State, Nigeria where about 60% are involved either as the sole means of employment or partly in order to meet up with the challenges of poor economy[29]. The use of herbicides is rampant both in the urban and rural population because of its application in agricultural practices and the control of undesired vegetation within the metropolis. Recent observations in many countries have been able to associate the declining rate of semen quality with herbicides which gain entrance into the human body either by environmental inhalation or by ingestion through contaminated water and food products[4]. The association between semen quality and herbicides which has been documented in many countries has not been evaluated in Ado-Ekiti hence this research work was carried out. The objective of this study is to assess the concentration of herbicide residues in seminal plasma samples from males with various sperm abnormalities (asthenospermia, oligospermia, azoospermia) compared to those with normal sperm parameters, their contributions to male infertility and the scientific evidence needed to justify the inclusion of herbicides determination as part of routine tests in the evaluation of male infertility.

2. METHODOLOGY

The aim of the study was to find out the role of some herbicides in the quality of semen produced by men whose wives were being evaluated for infertility.

2.1 Sample Collection Site

This research work was carried out in Ekiti State University Teaching Hospital, Ado-Ekiti, South western Nigeria. Geographically, the setting is located between latitude 7° 35 and 7° 47 north of equator and longitude 5° 11 and 5° 16 east of the Greenwich Meridian.[30]

2.2 Ethical Considerations

Ethical clearance with number EKSUTH/A67/2022/08/003 was obtained from the Research and Ethics unit of Ekiti State University Teaching Hospital before the commencement of the sample collection.

2.3 Selection of Subjects

Some men who were being evaluated for infertility and other male volunteers were included in the study after obtaining their consent in writing .Male volunteers who had torsion, varicocele , testicular damage and previous groin surgery were excluded from the study.

2.4 Sample Size

Based on the prevalence rate of 20-30%, the number of semen analysis evaluated in EKSUTH in the year 2022 was 400. Eighty samples accounting for 20% were selected out for the determination of herbicide residues. Equal numbers of 20 per group were randomly selected for the assessment of herbicides content for easy comparison in between the groups.

2.5 Sampling Procedures

The subjects were instructed to abstain from sexual intercourse for four to seven days after which semen samples were obtained from them by masturbation.

2.6 Semen Analysis Procedure

The samples were allowed to liquefy for 30 minutes before examination. Using the current 6th Edition WHO Laboratory manual[31], the following processes were carried out:

Physical Examination : Macroscopic examinations for volume, colour, viscosity and liquefaction were carried out on the semen samples.

Microscopic Examination : The sample was mixed well by inversion 3–5 times, and a drop was placed on the microscope stage and examined using a 10x objective lens and later a 40x high-power objective lens with a binocular microscope. The percentage motility, presence of agglutinates, debris, pus cells, bacteria and parasites such as Trichomonas vaginalis were all noted. Active sperm cells exhibit fast and directional straight movements. Sluggish sperm cells exhibits slower movements while non-motile or dead cells are motionless. By examining at least 10-20 fields using x40 objective, the percentage of each group was determined. Overall progressive movement is the sum of the active and sluggish cells.

Sperm Count : This was performed by diluting the specimen 1in 20 with 10% formol saline, followed by visual counting using the improved Neubaer counting chamber.

2.7 Criteria for Classification

Semen analysis were performed and the specimens were categorised into normospermic, asthenospermic, oligospermic and azoospermic groups based on the WHO criteria:[31]

Normospermia Samples: These were normal samples which were gray- white in colour, and were completely liquefied within 20 minutes of collection. The volume of each sample ranged between 1.5-5.0 mLs with the total percentage motility greater than 60%, without pus cells, red cells, bacteria nor parasites. The total sperm counts were also greater than 15 million/ml and the morphologically abnormal sperm cells were not more than 10%.

Asthenospermia: These were samples with reduced sperm motility and total progressives less than 32%. Samples with bacteria infections characterized by presence of pus cells and parasites were excluded.

Oligospermia: These were semen samples with normal motility and sperm counts less than 15.0 million per milliliter.

Azoospermia: These were semen samples with no sperm cells at all in the ejaculate confirmed by the examination of the centrifuged deposit. Clinical evaluations were carried out on the subjects to rule out those with physical blockage.

2.8 Selection of Samples for Biochemical Herbicides Assay

Twenty samples in each of the groups were selected out by simple convenient sampling technique, centrifuged hard at 3000rpm for 5 minutes to obtain the seminal plasma which were stored frozen till when their herbicide contents were measured.

2.9 Laboratory Procedure for Herbicides Assay

High Performance Liquid Chromatography method of analysis was used. Acetonitrile extraction/partitioning and dispense solid phase extraction process was carried out on the seminal plasma samples followed by injection into the HPLC system. The detection and quantitation was done using prepared standards at different wavelengths in the ultra violet region[32] To validate the procedures, intra assay measurement was done by analysing 20 aliquots of the same sample in the same run, and the mean, standard deviation and the coefficient of variation calculated. For the interassay measurement, each of the 20 aliquots were analysed in different runs on different days and the mean, standard deviation and co-

Table 1: Concentrations of Herbicides in the Seminal Plasma of Normospermic Men and Those With Asthenospermia

Herbicide	Normospermia	Asthenospermia	t-test	p-value	95% CI
	Mean ± SD (µg/g)	Mean ± SD (µg/g)			
Urea-Based And Branched-Chain Amino Acid-Inhibiting Herbicides					
Chlorimuron	8.68 ± 5.43	31.05 ± 13.91	-6.702	<0.01*	-29.12 - -15.61
Halosulfuron	2.89 ± 1.83	11.50 ± 6.65	-5.583	<0.01*	-11.73 - -5.49
Imaxamox	8.99 ± 5.82	31.33 ± 15.61	-5.997	<0.01*	-29.89 - -14.80
Cloransulam	1.86 ± 1.16	7.96 ± 5.49	-4.859	<0.01*	-8.64 - -3.56
Linuron	1.72 ± 1.11	7.35 ± 4.25	-5.730	<0.01*	-7.61 - -3.64
Fluometuron	16.91 ± 10.95	124.40 ± 193.90	-2.475	0.018*	-195.39 - -19.57
Synthetic Auxin-Based Herbicides					
Dicamba	2.34 ± 1.47	14.89 ± 20.49	-2.733	0.009*	-21.85 - -3.26
Fluroxypor	23.92 ± 14.82	77.00 ± 43.89	-5.125	<0.01*	-74.05 - -32.12
Trichlopyr	9.56 ± 6.43	32.87 ± 18.10	-5.428	<0.01*	-32.00 - -14.62
Propanil	7.45 ± 4.71	30.56 ± 16.31	-6.090	<0.01*	-30.80 - -15.43
Acetyl-CoA Carboxylase-Inhibiting Herbicides					
Clodinafop	1.70 ± 1.67	4.77 ± 2.27	-4.882	<0.01*	-4.35 - -1.80
Quizalofop	5.57 ± 3.67	23.03 ± 11.97	-6.235	<0.01*	-23.13 - -11.79
Clethodim	1.87 ± 1.18	6.22 ± 2.91	-6.205	<0.01*	-5.78 - -2.93
Fluazifop	4.88 ± 3.16	18.15 ± 8.02	-6.886	<0.01*	-17.18 - -9.37
Pinoxaden	2.01 ± 1.98	7.96 ± 6.95	-3.681	0.001*	-9.22 - -2.68
Photosynthesis-Inhibiting Herbicides					
Atrazine	7.02 ± 4.53	25.47 ± 11.52	-6.663	<0.01*	-24.06 - -12.85
Bentazon	1.94 ± 1.32	8.11 ± 4.18	-6.304	<0.01*	-8.16 - -4.19
Bromoxynil	5.43 ± 3.52	18.83 ± 9.53	-5.899	<0.01*	-18.00 - -8.80

*significant at $p < 0.05$; SD = standard deviation; CI = confidence interval (normospermia, n = 20; asthenospermia, n = 20)

Table 2: Herbicidal Concentrations in the Seminal Plasma of Normospermic and Oligospermic Men

Herbicide	Normospermia	Oligospermia	t-test	p-value	95% CI
	Mean ± SD (µg/g)	Mean ± SD (µg/g)			
Urea-Based And Branched-Chain Amino Acid-Inhibiting Herbicides					
Chlorimuron	8.68 ± 5.43	19.39 ± 14.03	-3.184	0.003*	-17.52 - -3.90
Halosulfuron	2.89 ± 1.83	16.70 ± 12.08	-3.746	0.002*	-14.08 - -3.86
Imaxamox	8.99 ± 5.82	10.39 ± 7.52	-0.659	0.514	-5.70 – 2.90
Cloransulam	1.86 ± 1.16	13.89 ± 10.05	-5.318	<0.01*	-16.61 - -7.45
Linuron	1.72 ± 1.11	11.25 ± 6.21	-6.763	<0.01*	-12.39 - -6.68
Fluometuron	16.91 ± 10.95	917.04 ± 268.52	-14.979	<0.01*	-1022.78 - -778.4
Synthetic Auxin-Based Herbicides					
Dicamba	2.34 ± 1.47	122.12 ± 64.17	-8.345	<0.01*	-148.83 - -90.72
Fluroxypor	23.92 ± 14.82	7.33 ± 4.05	4.827	<0.01*	9.63 – 23.54
Trichlopyr	9.56 ± 6.43	5.16 ± 2.85	2.802	0.008*	1.22 – 7.58
Propanil	7.45 ± 4.71	28.53 ± 26.34	-3.523	0.001*	-33.19 - -8.97
Acetyl-CoA Carboxylase-Inhibiting Herbicides					
Clodinafop	1.70 ± 1.67	5.37 ± 2.82	-5.012	<0.01*	-5.16 - -2.19
Quizalofop	5.57 ± 3.67	74.81 ± 39.31	-7.843	<0.01*	-87.11 - -51.37
Clethodim	1.87 ± 1.18	7.75 ± 4.07	-6.207	<0.01*	-7.80 - -3.97
Fluazifop	4.88 ± 3.16	55.67 ± 53.64	-4.227	<0.01*	-75.11 - -26.46
Pinoxaden	2.01 ± 1.98	36.83 ± 30.24	-5.140	<0.01*	-48.54 - -21.11
Photosynthesis-Inhibiting Herbicides					
Atrazine	7.02 ± 4.53	15.48 ± 8.54	-3.911	<0.01*	-12.83 - -4.08
Bentazon	1.94 ± 1.32	10.71 ± 5.91	-6.483	<0.01*	-11.51 - -6.03
Bromoxynil	5.43 ± 3.52	5.95 ± 3.28	-0.485	0.631	-2.70 - 1.66

*significant at $p < 0.05$; SD = standard deviation; CI = confidence interval, (normospermia, n = 20; azoospermia, n = 20)

efficient of variation determined. The intraassay and interassay for atrazine was 3.9% and 4.5% respectively.

2.10 Statistical Analysis

Statistical analysis was performed using the IBM SPSS Version 25. The means and standard deviations of the various herbicides were determined and T test was used to compare the normospermic separately with the asthenospermic, oligospermic and azoospermic groups while setting the confidence interval at 95% and the statistical significance at a P-value < 0.05[33].

2.11 Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available as they are yet to be deposited in a repository.

3. RESULT

3.1 Herbicides Concentrations In the Seminal Plasma of Normospermic and Asthenospermic Men

The concentrations of herbicides in the seminal plasma of normospermic and asthenospermic men are described in Table 1. Observations showed that in men with asthenospermia, all the four major classes of herbicides (urea-based and branched-chain amino-acid-inhibiting, synthetic auxin-based, acetyl CoA carboxylase -inhibiting and photosynthesis-inhibiting herbicides) had significantly higher seminal plasma concentrations than the values obtained in the men with normal sperm parameters.

3.2 Herbicidal Concentrations in the Seminal Plasma of Normospermic and Oligospermic Men

The concentrations of herbicides in the seminal plasma of normospermic and oligospermic men are shown in Table 2. From the table, higher levels of all the acetyl CoA carboxylase-inhibiting herbicides and photosynthesis-inhibiting herbicides (except bromoxynil) were significantly associated with oligospermia. The relationship between the synthetic auxin-based herbicides and oligospermia appeared to be equivocal because the concentrations of two types of the herbicides (dicamba and propanil) were significantly elevated while the other two (fluroxypor and trichlopyr) had significantly lower concentrations in the semen of oligospermic males when compared with the normospermic group. Most of the urea-based and branched-chain amino acid-inhibiting herbicides also had a significant relationship with oligospermia.

3.3. Concentrations of Herbicides in the Seminal Plasma of Normospermic and Azoospermic Men

Herbicidal concentrations in the seminal plasma of normospermic and azoospermic men are shown in Table 3. When compared with normospermic males, men with azoospermia had significantly elevated concentrations of herbicides from all the major classes. The quantities of chlorimuron, imaxamox and trichlopyr in both normospermic and azoospermic groups of participants were comparable.

3.4 Total Detectable Herbicides in the Seminal Plasma of all the Groups

Figure 1 shows the illustration (histogram presentations) of the overall concentrations of the total herbicides in the seminal plasma of the respondents. The total detectable herbicides in the oligospermic group is the highest followed by that of the azospermic group while that of the normospermic group is the least.

4. DISCUSSION

Impairment of spermatogenesis and sperm function is the most common cause of male infertility[3]. From this study, the concentrations of most herbicides in the seminal plasma were significantly higher in the abnormal sperm groups (i.e asthenospermic, oligospermic, and azoospermic) subjects compared with the normospermic group showing that they are associated with poor semen quality.

Asthenospermia is a condition which is characterized by poor sperm motility. From the results of this study, the concentration of all the various of herbicides listed were significantly increased in

Table 3: Herbicidal Concentrations In The Seminal Plasma Of Normospermic And Azoospermic Men

Herbicide	Normospermia	Azoospermia	t-test	p-value	95% CI
	Mean ± SD (µg/g)	Mean ± SD (µg/g)			
Urea-Based And Branched-Chain Amino Acid-Inhibiting Herbicides					
Chlorimuron	8.68 ± 5.43	13.78 ± 10.61	-1.855	0.073	-10.67 – 4.94
Halosulfuron	2.89 ± 1.83	11.86 ± 9.14	-3.746	0.002*	-14.08 - -3.86
Imaxamox	8.99 ± 5.82	7.38 ± 5.69	0.816	0.420	-2.40 – 5.61
Cloransulam	1.86 ± 1.16	9.87 ± 7.60	-4.045	0.001*	-12.24 - -3.77
Linuron	1.72 ± 1.11	15.90 ± 4.63	-13.247	<0.01*	-16.36 - -12.00
Fluometuron	16.91 ± 10.95	715.95 ± 200.52	-15.638	<0.01*	-789.98 - -608.09
Synthetic Auxin-Based Herbicides					
Dicamba	2.34 ± 1.47	147.79 ± 48.55	-13.459	<0.01*	-167.44 - -123.47
Fluroxypor	23.92 ± 14.82	10.36 ± 3.02	3.476	0.001*	5.62 – 21.49
Trichlopyr	9.56 ± 6.43	7.29 ± 2.12	1.313	0.198	-1.25 – 5.79
Propanil	7.45 ± 4.71	28.28 ± 22.70	-3.500	0.003*	-33.54 - -8.14
Acetyl-CoA Carboxylase-Inhibiting Herbicides					
Clodinafop	1.70 ± 1.67	6.50 ± 2.13	-7.479	<0.01*	-6.11 - -3.50
Quizalofop	5.57 ± 3.67	90.53 ± 29.74	-12.713	<0.01*	-98.57 - -71.37
Clethodim	1.87 ± 1.18	9.38 ± 3.08	-10.014	<0.01*	-9.04 - -5.99
Fluazifop	4.88 ± 3.16	115.36 ± 80.25	-6.182	<0.01*	-146.84 - -74.12
Pinoxaden	2.01 ± 1.98	69.71 ± 48.49	-6.268	<0.01*	-89.67 - -45.72
Photosynthesis-Inhibiting Herbicides					
Atrazine	7.02 ± 4.53	21.87 ± 6.37	-8.061	<0.01*	-18.59 - -11.10
Bentazon	1.94 ± 1.32	15.14 ± 4.41	-12.703	<0.01*	-15.31 - -11.08
Bromoxynil	5.43 ± 3.52	8.41 ± 2.45	-2.806	0.008*	-5.14 - -0.82

*significant at $p < 0.05$; SD = standard deviation; CI = confidence interval .
(normospermia, n = 20; azoospermia, n = 20)

asthenospermic subjects compared with the normospermic subjects. One of the mechanisms by which herbicides cause asthenospermia is through induction of oxidative stress in the testes leading to sperm cells damage. Linuron, halosulfuron, fluroxypyr, bromoxynil, and fluasifol have been shown to induce oxidative stress in previous studies. The findings in this study agrees the reports of Prothina et al[13], Kim et al[14], Ore and Olayinka[15], and El-Nagar and Elsisi[16]. Oxidative stress is the result of imbalance between the production of reactive oxygen species (ROS) and the counter mechanisms of the antioxidants. Since mammalian spermatozoa membrane is rich in polyunsaturated fatty acids which provides the plasma membrane with the fluidity essential at fertilization, they are highly susceptible to oxygen-induced damage often mediated by lipid peroxidation[34]. Exposure to increased ROS concentration leads to lipid peroxidation, DNA damage, protein oxidation which in turn affects sperm motility. Oxidative stress also disrupts the functions and structure of the flagellum, the organelle responsible for sperm motility by damaging the microtubules and dynein axons of the flagellum resulting in asthenospermia[35]. ROS generation triggers off a cascade of mechanism in the testes culminating in the development of asthenospermia. It also interferes with the calcium signaling pathways which regulate flagella beating and sperm motility[36].

In addition, many of the herbicides are endocrine disruptors, affecting the hypothalamus, pituitary-testicular axis. The findings in this study is supported by previous reports, of Hayes et al [37]. For, example, in this study, higher concentration of Atrazine is associated with the development of asthenospermia. Atrazine has been implicated in the disruption of hypothalamus pituitary gonadal axis, leading to poor sperm motility[10,38]. In the same vein, our observation about dicamba in this study is supported by that of Zhu et al[9], and Sengueta et al[11].

Furthermore, some herbicides have been found to directly affect the structure and function of sperm cells both of which are aetiological factors in the development of asthenospermia. For example, paraquat has been shown to disrupt the mitochondrial membrane potential in sperm cells, leading to decreased motility[36]. Mitochondria play a crucial role in providing energy for sperm motility, and any disruption in their function can lead to impaired sperm movement. Herbicides also induce oxidative stress in the testes, which can damage mitochondrial DNA and proteins, affecting their ability to produce ATP and support sperm motility[39].

Another explanation to the findings in this study is that some herbicides interferes with calcium signalling, sperm chemotaxis and acrosome reactions culminating in asthenospermia. Klinefelter et al[40] had demonstrated earlier that atrazine exposure altered intracellular calcium levels in sperm cells leading to asthenospermia. Herbicides can also interfere with sperm chemotaxis, which is the ability of sperm cells to navigate towards an egg cell using chemical signals[41] and interfere with acrosome reaction which is a crucial step in the fertilization process where enzymes are released from the acrosome of the sperm cell to penetrate the egg's protective layer[36].

Oligospermia is a condition characterised by low sperm count in the ejaculate, usually below 15 million sperm cells per ml indicating a decrease in spermatogenesis output[31]. This study also show that most of the urea based herbicides, branch chain amino acid inhibiting herbicides, synthetic auxin herbicides, acetyl-CoA carboxylase inhibiting herbicides and photosynthesis inhibiting herbicides were positively correlated with the development of oligospermia. Some of the causes of oligospermia as documented in literature includes endocrine dysfunctions, enviromental toxins, and anatomic abnormalities[42]. Hypogonadism and hormonal imbalance has been shown to lead to partial spermatogenic arrest, low levels of testosterone and increased estrogen in the male all of which are contributing factors towards the pathogenesis of oligospermia[43]. Herbicides such as atrazine, dicamba, fluroxypyr,and bromoxynil have been implicated as endocrine distruptors in previ-

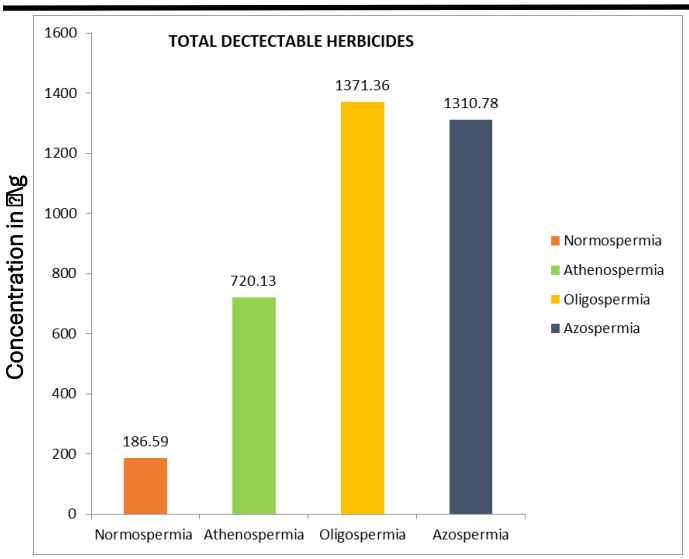

Figure 1: Total Detectable Herbicides In The Respondents

ous studies[9,10,11]. Testosterone, is the principal male hormone which is essential for spermatogenesis and low levels lead to oligospermia.[44] Our findings in this study shows that atrazine is associated with significant increase in the level of oligospermia and it agrees with earlier reports of Swan[45] and Betancount et al[46]. This is because Atrazine disrupts the normal hypothalamic control of pituitary-ovarian function as well as the testicular axis and stimulates the metabolic activities for aromatase enhancing the conversion of testosterone to oestrogen thus promoting feminism and preventing masculinization[46,47]. Trichlopyr is also a known endocrine disruptor that affects thyroid metabolism[48], and thyroid dysfunction in turn causes alterations in testicular functions and semen quality[49]. Our observation about dicamba in this study agrees with the report of Zhu et al[9]. In addition, propanil have similar effects with estrogen in the immune system showing that it mimics the natural hormone thus buttressing the fact that propanil action induces feminism in the body which in turn negatively affects spermatogenesis[50]. This research support the findings of Nowak et al[51] and Kongtip et al[52].

Azoospermia is a condition in which there is no sperm cell found in the semen ejaculate collected at two to three different times more than two weeks apart even after the specimen must have been centrifuged and the deposit examined[31]. The condition could be due to destruction of the germinal epithelial cells, varicocele, obstruction in the male reproductive paths, hormonal diseases such as hypogonadism and pituitary gland disorders, Kallmann's or Klinefelter's syndrome and gynecomastia[53]. In this study, many of the herbicides were significantly increased in azoospermic subjects compared with the the normospermic. The mechanism by which herbicides could lead to the development of azoospermia are endocrine disruption, destruction of germinal cells, DNA damage, meiotic arrest and induction of oxidative stress[53]. The mechanism by which herbicides disrupt the normal rhythm of the hypothalamus- pituitary-gonadal axis has been discussed above. In addition to the properties of these herbicides as endocrine disruptors, some of them also exhibit genotoxic effects on the testicular cells, causing DNA fragmentation and induction of hermaphroditism[36]. This is supported by the study of Komsky-Elbaz et al[54]. While there is paucity of information as to the association of many of these individual herbicides with the development of azoospermia in literature, our findings in this study about atrazine is supported by the reports of Omran et al[55] which demonstrated that atrazine exposure causes azoospermia in snails by destroying the testicular cells. The results from our study indicated that dicamba is strongly associated with azoospermia. This is corroborated by previous studies which reported that dicamba exposure is associated with testicular damage, DNA damage and inhibition of spermatogenesis.[9] Trichlopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) interferes with cell divisions and growth in plants leading to impairment of vascular transport and death[56] and these properties are antagonists in spermatogenesis. It has been shown to have toxic effects on Zebra fish embryos[57]. Immazamox also caused apoptotic changes in rat liver and pancreas[58,59]. Such apoptotic changes could lead to oligospermia and azoospermia. It affects corticosteroids metabolism[60]. It could also affect testosterone metabolism since it is a steroid like Cortisol[61,59].

Some of the herbicides are also enzyme inhibitors. The synthesis of amino acids in adequate amounts are important in cell divisions which is germane to the process of spermatogenesis[62]. Herbicides that inhibits the synthesis of branched chain amino acids are chlorimurion, halosulfuron, imaxamox, chloramsulam, diuron, linuron and fluometuron[7], and most of them are positively correlated with azoospermia in this study showing that their roles as antagonists in protein synthesis could be an underlying factor in the pathogenesis of azoospermia. This is supported by the reports of Hou et al.[63] Members of this group, inhibit acetolactate synthase, an enzyme that catalyzes the first step in the biosynthesis of branched chain amino acids which are valine, isoleucine, and leucine. Branched chain amino acids have been implicated to play important role in male reproductive performance. For example, long treatment of leucine and one of its metabolites β-hydroxy β-methyl butyrate have been reported to increase the concentrations of insulin-like growth factor 1 (IGF1) and insulin in rats[64]. Both of these i.e IGF-1 and insulin play a major role in testicular function such as maintaining the normal number of Leydig cells, maturation of these cells and steroidogenesis. Also, a metabolic end product of leucine i.e β-hydroxyl –β- methyl glutaryl coenzyme A is the precursor of cholesterol synthesis, cholesterol is a precursor in the synthesis of testosterone which is the main hormone necessary for male reproductive performance. It has also been reported that leucine supplementation stimulates leptin secretion which also enhances release of testosterone from the testes. This implies that inhibition of the synthesis of these branched chain amino acids will hinder testicular functions and spermatogenesis hence the observations made in this study[64].

Clodinafop, quizalofop, clethodim, fluazifop and pinoxadem are acetyl-CoA carboxylase-inhibiting herbicides which interfere with lipid metabolism. Acetyl CoA carboxylase (ACCase) is the enzyme that catalyzes the first step in fatty acid biosynthesis. These ACCase inhibiting herbicides, therefore block this first step and thus prevent the formation of fatty acids required for phospholipid synthesis which are important components of the cell membranes of sperm cells. This will lead to a loss of cell membrane integrity, metabolite leakage and apoptosis[65]. The observation in this study is in agreement with EFSA reports of 2018[24] that links Clodinafop, with reduced testicular weight, cellular destruction, endocrine disruption and reduced spermatogenesis[24]. It was also reported that Clethodim effects on rat testis include testicular damage, testicular atrophy, decreased testosterone synthesis and decreased amount of germ cells which are responsible for sper-

matogenesis[22]

The association of some herbicides with azoospermia as observed in this study could also be due to meiotic arrest as reported in previous studies[66]. Furthermore, lack of Boule protein expression has been identified as a cause of azoospermia in men[67] and lower expression of the Boule protein has been observed in herbicide poisoning[66].

Moreover, herbicides can induce oxidative stress which is also one of the causes of azoospermia[68]. Some herbicides such as atrazine decreases antioxidants actions thus promoting oxidative stress, the process which leads to azoospermia. Recently, Bautista et al, demonstrated that atrazine decreases sperm and antioxidants quality in the testis of Zebra fish[69]. In summary, all these properties exhibited by these herbicides can culminate in the development of azoospermia.

4.1 Conclusion

In this study, we conclude that exposure to these herbicides could be one of the causes of male infertility in Ado-Ekiti since its an agrarian community. It is recommended that routine screening of infertile men should include the determination of herbicides in seminal plasma and appropriate therapy should be administered. It is also suggested that appropriate legislation be enacted regarding the use of herbicides in order to safeguard public health. Furthermore, there is need for advocacy and emphasis on wearing of personal protective equipment for farmers.

Conflicts of Interest

The authors declare no conflicts of interest

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Ajayi, D.D: Conceptualization, Analysis, Resources, Methodology, Visualization, Literature search, Original draft writing , Supervision & Writing - review and editing .
Awoleke J.O: Risk of bias assessment, Initial statistics execution & Writing - review and editing .
Adewara E.O: Logistical planning, Allocation of duties, Validation & Writing - review and editing .
Ajayi, O.B: Draft review & Editing of final manuscript & Writing - review and editing .
Ajayi, O.S: Laboratory investigation, Resources & Writing - review and editing

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5. REFERENCE

1. De Jonge C, Barratt C L R. The present crisis in male reproductive health: an urgent need for a political, social, and research roadmap. Andrology 2019; 7(6), 762–768.
2. Hossain F, Ali O, D'Souza U J, Naing D K. Effects of pesticide use on semen quality among farmers in rural areas of Sabah, Malaysia. Journal of occupational health. 2010; 52 (6), 353–360.
3. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018;62:2-10. d
4. Nateghian Z, Aliabadi E. Aspects of Environmental Pollutants on Male Fertility and Sperm Parameters. J Environ Treat Tech 2020;8(1):299–309.
5. Sciorio R, Tramontano L, Adel M, Fleming S. Decrease in Sperm Parameters in the 21st Century: Obesity, Lifestyle, or Environmental Factors? An Updated Narrative Review. Journal of personalized medicine 2024; 14(2), 198.
6. Stehle S, Bline A, Bub S, Petschick LL, Wolfram J, Schulz R, et al. Aquatic pesticide exposure in the U.S. as a result of non-agricultural uses. Environ Int. Dec 2019;133(Pt B):105234.
7. Ross, MA, Childs DJ. Herbicide Mode-of-Action Summary. Purdue University, Department of Botany: Plant Pathology, West Lafayette IN. Report No. WS-23-W. 1996.
8. de Araújo-Ramos AT, Passoni MT, Romano MA, Romano, RM, Martino-Andrade AJ, et al. Controversies on Endocrine and Reproductive Effects of Glyphosate and Glyphosate-Based Herbicides: A Mini-Review. Front Endocrinol (Lausanne). 2021;12:627210.
9. Zhu L, Li W, Zha J, Wang, Z, et al. Dicamba affects sex steroid hormone level and mRNA expression of related genes in adult rare minnow (Gobiocypris rarus) at environmentally relevant concentrations. Environ Toxicol. 2015;30(6):693-703.
10. Trentacoste SV, Friedmann AS, Youker RT, Breckenridge CB, Zirkin BR, et al. Atrazine effects on testosterone levels and androgen-dependent reproductive organs in peripubertal male rats. J Androl. 2001;22(1):142-8.
11. Sengupta P, Banerjee R. Environmental toxins: Alarming impacts of pesticides on male fertility.Human & Experimental Toxicology. 2014;33(10):1017-1039.
12. Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi, K, et al. Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ. Health Perspect. 2004;112, 524–531.
13. Prathima P, Venkaiah K, Daveedu T, Pavani R, Sukeerthi S, Gopinath M, et al. α-lipoic acid protects testis and epididymis against linuron-induced oxidative toxicity in adult rats. Toxicol Res. 2020;36(4):343-357.
14. Kim M, An G, Lim W, Song G, et al., Fluroxypyr-1-methylheptyl ester induced ROS production and mitochondrial apoptosis through the MAPK signaling cascade in porcine trophectoderm and uterine luminal epithelial cells. Pestic Biochem Physiol. 2022;187:105196
15. Ore A, Olayinka ET. Fluazifop- p-butyl, an aryloxyphenoxypropionate herbicide, diminishes renal and hepatic functions and triggers testicular oxidative stress in orally exposed rats. Toxicol Ind Health. 2016;33(5):406-415.
16. El-Nagar MF, Elsisi AE. Exposure to bromoxynil octanoate herbicide induces oxidative stress, inflammation, and apoptosis in testicular tissue via modulating NF-κB path-

way. Food Chem Toxicol. 2023

17. Taşkıran M. Is There an Association Between Dietary Antioxidant Levels and Sperm Parameters in Male Infertility?. Cureus 2023; 15(8), e44339. https://doi.org/10.7759/cureus.44339

18. Chyra-Jach D, Kaletka Z, Dobrakowski M, Machoń-Grecka A, Kasperczyk S, Birkner E, Kasperczyk A. The Associations between Infertility and Antioxidants, Proinflammatory Cytokines, and Chemokines. Oxidative medicine and cellular longevity; 2018, 8354747.

19. Zhu H, Wang H, Cheng Y, Liu D, Zhang A, Wen Z, Gao,J. Hadh deficiency induced oligoasthenoteratozoospermia through the TNF-α/Bcl-2 pathway in male mice. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2022: 36(12).

20. Ventimiglia E, Viganò P, Alfano M, Abbate C, Cornelius J, Mattei A, Montorsi F, Salonia A. Correlation among isolated teratozoospermia, sperm DNA fragmentation and markers of systemic inflammation in primary infertile men. PloS one, 2021;16(6), e0251608. https://doi.org/10.1371/journal.pone.0251608

21. McIntyre BS, Barlow NJ, Foster PM, et al. Male rats exposed to linuron in utero exhibit permanent changes in anogenital distance, nipple retention, and epididymal malformations that result in subsequent testicular atrophy. Toxicol Sci. 2002;65(1):62-70.

22. Dcunha R, Kumari S, Najar MA, Aravind A, Suvarna KS, Hanumappa A, et al. High doses of clethodim-based herbicide GrassOut Max poses reproductive hazard by affecting male reproductive function and early embryogenesis in Swiss albino mice. 2023;336:139215.

23. EFSA Scientific Committee. Scientific Opinion on the hazard assessment of endocrine disruptors: scientific criteria for identification of endocrine disruptors and appropriateness of existing test methods for assessing effects mediated by these substances on human health and the environment. 2013;11(3):3132.

24. European Food Safety Authority (EFSA); Arena M, Auteri D, Barmaz S, Brancato A, Brocca D, Bura L, et al. Peer review of the pesticide risk assessment of the active substance clodinafop (variant evaluated clodinafop-propargyl). 2018;16(11):e05467.

25. Shen Y, Zhang J, Xie J, Liu J, et al. In vitro assessment of corticosteroid effects of eight chiral herbicides. J Environ Sci Health 2019;55(2):91-102.

26. Zhang Y, Zhang J, Shi B, Li B, Du Z, Wang J, et al. Effects of cloransulam-methyl and diclosulam on soil nitrogen and carbon cycle-related microorganisms. Journal of Hazardous Materials 2021;418

27. Meftaul IM, Venkateswarlu K, Dharmarajan R, Annamalai P, Asaduzzaman M, Parven A, et al. Controversies over human health and ecological impacts of glyphosate: Is it to be banned in modern agriculture? Environ Pollution. 2020;263(Pt A):114372.

28. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond B Biol Sci; 2010;365 (1546):1697–1712.

29. Kolawole OD, Okorie VO, Ogidiowa MT, Adeogun MO. Ethno-veterinary practices amongst small-holder farmers in Ekiti state, Nigeria. Afr J Tradit Complement Altern Med; 2007 10;4(4):434-42.

30. Olayanju AO, Chris NI, Emmanuel AE, Oyetunde AB, Adebisi KY, Okolo CS. Prevalence of alloantibodies associated with haemolytic disease of the fetus and newborn in pregnant women at the Ekiti State University Teaching Hospital, Ado-Ekiti, Southwest Nigeria. Afr J Reprod Health. 2023 27(6s):70-78.

31. World Health Organisation. WHO laboratory manual for the examination and processing of human semen, Sixth Edition 2021. ISBN 978-92-4-003078-7 (electronic version).

32. Johnson CL, Jazan E, Kong SW, Pennell KD, et al. A two-step gas chromatography-tandem mass spectrometry method for measurement of multiple environmental pollutants in human plasma. Environ Sci Pollut Res Int 2020;28 (3):3266-3279.

33. IBM Corp. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp; 2016

34. Wathes DC, Abayasekara DR, Aitken DJ, et al. Polyunsaturated fatty acids in male and female reproduction. Biology of reproduction, 2007;77(2):190-201.

35. Storey BT. Biochemistry of the induction and prevention of the lipoperoxidative damage in human spermatozoa. Mol. Tium Reprod 1997;3:203-214.

36. Kumar, N. “Sperm Mitochondria, the Driving Force Behind Human Spermatozoa Activities: Its Functions and Dysfunctions - A Narrative Review.” Current molecular medicine vol. 23,4 2023: 332-340.

37. Hayes T B, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proceedings of the National Academy of Sciences of the United States of America vol. 2002;99 (8) 5476-80.

38. Ikeji CN, Adedara IA, Farombi EO. Dietary myricetin assuages atrazine-mediated hypothalamic-pituitary-testicular axis dysfunction in rats. Environ Sci Pollut Res Int. 2023;30(6):15655-15670.

39. de Liz Oliveira Cavalli VL, Cattani D, Heinz Rieg CE, Pierozan P, Zanatta L, Benedetti Parisotto E, Wilhelm Filho D, Mena Barreto Silva FR, Pessoa-Pureur R, Zamoner A. Roundup disrupts male reproductive functions by triggering calcium-mediated cell death in rat testis and Sertoli cells. Free Radical Biology and Medicine;2013; 65:335-346

40. Klinefelter GR, Strader LF, Suarez JD, Roberts NL. Changes in calcium levels in rat testicular cells after atrazine treatment. Reprod Toxicol. 2006;22(4):584-591

41. Guidobaldi HA, Teves ME, Uñates DR, Anastasía A, Gi-ojalas LC. Progesterone from the cumulus cells is the sperm chemoattractant secreted by the rabbit oocyte cumulus complex. PLoS One. 2017;12(5):e0171693

42. Choy J T, Amory J K. Nonsurgical Management of Oligozoospermia. The Journal of clinical endocrinology and metabolism 2020;1;105(12):e4194–207

43. Colpi G M, Francavilla S, Haidl G, Link K, Behre H M, Goulis D G, Krausz C, Giwercman A. European Academy of Andrology guideline Management of oligo-asthenoteratozoospermia. Andrology 2018; 6(4), 513–524.

44. Liu H, Zheng H, Li Y, Tang Y, Peng H, Li Q, Zhuang J, Zhou Y, Zhou, Y, Tu X, Zhang X. Seminal testosterone, rising viewpoint of local spermatogenesis in nonobstructive azoospermia: One center long-term bidirectional cohort study. Frontiers in endocrinology 2022) 13, 992556.

45. Swan SH. Semen quality in fertile US men in relation to geographical area and pesticide exposure. Int J Androl 2006; 29: 62–68.

46. Betancourt M, Resendiz A, Casas E and Fierro R(2006) “Effects of two insecticides and two herbicides on the porcine sperm motility patterns using computer associated semen analysis (CASA) in vitro “ Reproductive toxicology 22(3) 508-512.

47. Hayes T B, Anderson L L, Beasley V R, de Solla S R, Iguchi T, Ingraham H et al. Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. The Journal of steroid biochemistry and molecular biology 2011; 127(1-2), 64–73.

48. Zhang J, Begum A, Brännström K, Grundström C, Iakovleva I, Olofsson A, Sauer-Eriksson AE, Andersson PL. Structure-Based Virtual Screening Protocol for in Silico Identification of Potential Thyroid Disrupting Chemicals Targeting Transthyretin. Environ Sci Technol. 2016 Nov 1;50(21):11984-11993.

49. La Vignera S, Vita R. Thyroid dysfunction and semen quality. Int J Immunopathol Pharmacol. 2018 Jan-Dec;32:2058738418775241.

50. Salazar KD, Miller MR, Barnett JB, Schafer R. Evidence for a novel endocrine disruptor: the pesticide propanil requires the ovaries and steroid synthesis to enhance humoral immunity. Toxicol Sci. 2006; 93(1):62-74.

51. Nowak K, Jabłońska E, Ratajczak-Wrona W. Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. Environ Int. 2019 125:350-364.

52. Kongtip P, Nankongnab N, Kallayanatham N, Pundee R, Choochouy N, Yimsabai J, Woskie S. Thyroid Hormones in Conventional and Organic Farmers in Thailand. Int J Environ Res Public Health. 2019; 29;16(15):2704.

53. Cocuzza, M., Alvarenga, C., & Pagani, R. (2013). The epidemiology and etiology of azoospermia. Clinics (Sao Paulo, Brazil), 68 Suppl 1(Suppl 1), 15–26. https://doi.org/10.6061/clinics/2013(sup01)03

54. Komsky-Elbaz A, Kalo D, Roth Z. (2021). Carryover effect of atrazine and its metabolite-from treated bovine spermatozoa to the embryo's transcriptome†. Biology of reproduction; 104(5), 1162–1180.

55. Omran N E, Salama W M. The endocrine disruptor effect of the herbicides atrazine and glyphosate on Biomphalaria alexandrina snails. Toxicology and industrial health 2016; 32(4), 656–665.

56. Ahrens WH. Herbicide handbook. Seventh Edition 1994. [Google Scholar] [Ref list

57. Suvarchala G, and Philip GH. Toxicity of 3,5,6-trichloro-2-pyridinol tested at multiple stages of zebrafish (Danio rerio) development. Environ Sci Pollut Res Int. 2016 23 (15):15515-23.

58. Sevim Ç, Çomaklı S, Taghizadehghalehjoughi A, Özkaraca M, Mesnage R, Kovatsi L, Burykina TI, Kalogeraki A, Antoniou MN, Tsatsakis A. An imazamox-based herbicide causes apoptotic changes in rat liver and pancreas. Toxicol Rep. 2018 ; 19;6:42-50.

59. Jin J, Kurobe T, Ramírez-Duarte WF, Bolotaolo MB, Lam CH, Pandey PK, Hung TC, Stillway ME, Zweig L, Caudill J, Lin L, Teh SJ. Sub-lethal effects of herbicides penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (Hypomesus transpacificus). Aquat Toxicol. 2018 ;197:79-88. doi: 10.1016/j.aquatox.2018.01.019. Epub 2018 Feb 1. PMID: 29448126.

60. Shen Y, Zhang J, Xie J, Liu J. In vitro assessment of corticosteroid effects of eight chiral herbicides. J Environ Sci Health B. 2020;55(2):91-102.

61. Tsatsakis A, Tyshko NV, Docea AO, Shestakova SI, Sidorova YS, Petrov NA, Zlatian O, Mach M, Hartung T, Tutelyan VA. The effect of chronic vitamin deficiency and long term very low dose exposure to 6 pesticides mixture on neurological outcomes - A real-life risk simulation approach. Toxicol Lett. 2019; 15;315:96-106. doi: 10.1016/j.toxlet.2019.07.026. Epub 2019 Aug 3. PMID: 31386889.

62. Dai Z, Wu Z, Hang S, Zhu W, Wu G. Amino acid metabolism in intestinal bacteria and its potential implications for mammalian reproduction. Molecular human reproduction. 2015; 21(5), 389–409.

63. Hou Y, Yao K, Yin Y, Wu G. Endogenous Synthesis of Amino Acids Limits Growth, Lactation, and Reproduction in Animals. Advances in nutrition (Bethesda, Md.) 2016 7(2), 331–342.

64. Storey B T. Biochemistry of the induction and prevention of the lipoperoxidative damage in human spermatozoa. Mol. Tium Reprod 1997; 3: 203-214.

65. Kaundun, S S. Resistance to acetyl CoA carboxylase inhibiting herbicides. Pest Management Science 2014; 70(9), 1405-1417.

66. Ahmadivand S, Farahmand H, Teimoori-Toolabi L, Mirvaghefi A, Eagderi S, Geerinckx T, Shokrpoor S, Rahmati-Holasoo H. Boule gene expression underpins the meiotic arrest in spermatogenesis in male rainbow trout

(Oncorhynchus mykiss) exposed to DEHP and butachlor. General and comparative endocrinology,2016; 225, 235–241.

67. Luetjens C M, Xu EY, Rejo Pera R A, Kamischke A, Nieschlag E, Gromoll J. Association of meiotic arrest with lack of BOULE protein expression in infertile men. The Journal of clinical endocrinology and metabolism. 2004; 89(4), 1926–1933.

68. Le J, Lei X, Ren Y, Li Z, Tu H, Ding F, Yi X, Zhou Y, Liu Q, Zhang S. Exogenous oestradiol benzoate induces male mice azoospermia through modulation of oxidative stress and testicular metabolic cooperation. Molecular medicine reports 2019; 19(6), 4955–4963.