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Investigation of single strand DNA breaks in spontaneous abortion cases with known pesticide exposure

Jale Akgol[1], Mine Kanat Pektas[2]

[1]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Pharmacology, Afyonkarahisar, Türkiye*
[2]*Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Obstetrics and Gynecology, Afyonkarahisar, Türkiye*

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Abstract

Pesticide exposure is an essential public health problem and endocrine-disrupting factor. Encounters during pregnancy may cause spontaneous abortions or congenital anomalies with embryotoxic or fetotoxic effects. DNA double-strand breaks occur upon exposure of DNA to radiation and chemicals or are caused by faulty DNA metabolic processes. This research examines the relationship of DNA single-strand amount with pesticides and their types. Single-stranded DNA (ssDNA) density was quantitatively determined by fluorometric method in the blood of Spontaneous Abortion with organochlorine and or organophosphate pesticide exposure and in the blood of two patient groups who had normal delivery without pesticide contact. The mutation was screened by next-generation sequencing in 4 women who experienced spontaneous abortion with a high pesticide amount and variety. 91 female patients aged 18-35 years were included in the study. The spontaneous abortion group was 56 (61.5%), and the normal delivery group was 35 (38%) women. The mean age was 28.18(±4.5). There was a significant correlation between Pentachlorophenol -52 (PCB52) and ssDNA level ($p<0.05$). There was no relationship between ssDNA ratios and groups ($p>0.05$). It was determined that ssDNA levels were higher among those with multiple abortions. The ssDNA level was higher in those who had an abortion before ($p<0.05$). We determined that dichloro di phenyl tri chloro ethane (DDT) and its congeners were significantly higher in the Whole Exome Sequence analysis of 2 of the four patients with the highest pesticide level. The DYNC2H1 gene NM_001377.3 c.7506G>A (p.Trp2502Ter) variation and the DYNC2H1 gene NM_001377.3 c.8458C>A (p.Pro2820Thr) variation associated with recurrent miscarriages were found to be heterozygous. Studies in this area need to be increased to determine the relationship of ssDNA elevation with pesticide exposure, its predictive value in abortion, and the value of environmental factors in repeated abortions whose etiology has not been clarified.

Keywords: Pesticide, spontaneous abortion, amount of ssDNA, genotoxicity, mutation

Introduction

Spontaneous Abortion (SAB) is a complication of pregnancy with multifactorial etiology. It is common in 10-15% of clinical pregnancies. Anatomical, genetic, endocrine, immunologic, or environmental factors affecting the mother, placenta, or fetus are responsible for spontaneous abortion. Although most are associated with cytogenetic pathologies of the embryo, 50-75% of cases are not fully elucidated. Spontaneous abortion, often thought to be inevitable, can be prevented, especially in recurrent cases when the causes are identified and treated [1,2].

The relationship between pesticides and reproductive toxicity has been revealed in many studies. Especially studies conducted with women working in agricultural fields support the hypothesis that there is a relationship between pesticides and SAB [3,4]. For example, the role of dichloro di phenyl tri chloro ethane (DDT) exposure in spontaneous pregnancy loss has been previously reported [5]. DNA double-strand breaks occur upon exposure of DNA to radiation and chemicals or are caused by faulty DNA metabolic processes. The resulting single-stranded DNA is a form in which the double helix structure of the DNA molecule

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Corresponding Author: Jale Akgol, Afyonkarahisar Health Sciences University Faculty of Medicine, Department of Medical Pharmacology, Afyonkarahisar, Türkiye
Email: jaleakgol@gmail.com

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consists of a single chain of nucleotides (ssDNA). High plasma ssDNA levels are an indicator of genomic instability [6]. This study examines whether there is a relationship between pesticide levels and DNA breaks in a group of patients.

Material and Methods

Study design

This study is a case-control study. In this study, we investigated the significance of ssDNA level differences between a group of women who had spontaneous pregnancy loss in whom various pesticide residues were found in their blood by chromatographic methods and women who had a normal delivery with no pesticides in their blood. We decided on the Sample Size with the G-Power 3.1 analysis. When the study effect size was 0.80, the alpha error rate was 0.05, and the group ratio to be compared was 1.6/1 for 95% power. Fifty-four patients and 34 control groups were required. We included 56 patients (61.5%) and 35 controls (38.5%) in the study group [7]. The characteristics of the study and control groups are shown in Table 1.

Table 1. Study and control group inclusion criteria

Study group inclusion criteria	Control group inclusion criteria
• 18 to 35 years old,	• 18 to35 years old
• Spontaneous termination of pregnancy within the first 20 weeks of pregnancy and followed up in hospital	• A timely and healthy termination of pregnancy
• No chronic diseases (diabetes, hypertension, thyroid disease)	• No chronic disease (Diabetes, Hypertension, Thyroid disease)
• Does not smoke, does not drink alcohol	• Non-smoker, non-drinker
• No consanguineous marriage	• No consanguineous marriage
• Spontaneous abortions that do not occur due to cervical insufficiency	• Not pregnant as a result of treatment
• No chromosomal abnormalities were detected in the cytogenetic analysis of the abortion material	• No exposure detected as a result of pesticide analysis
• No history of drug exposure leading to abortion	• Allowing genetic screening
• Not pregnant through treatment	
• In pesticide analysis results, at least one exposure detection was detected in pesticide quantity analysis	
• Allowing genetic screening	

Study selection flowcart is in Figure 1. We screened abortion-related genotoxicity using Next Generation Sequencing in four samples that were found to have multiple and high exposure to organochlorine and organophosphate pesticide compounds, which were determined by LC-MS/MS (Tandem mass spectrometry-especially in combination with liquid chromatography) and GC-MS/MS (gas chromatography-tandem mass spectrometry) in the previous study.

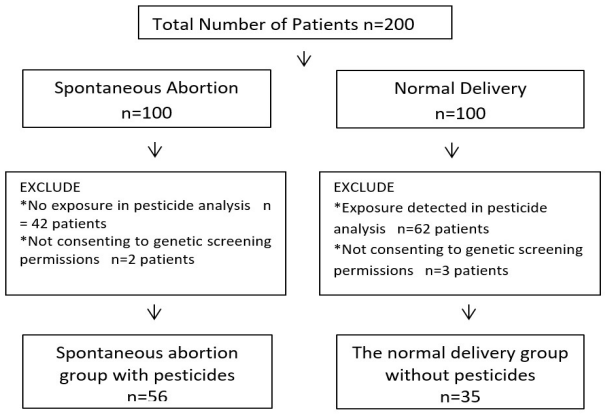

Figure 1. Study selection flowcart

Sample collection

The blood samples were obtained from the 'Determination of the Relationship Between Spontaneous Abortion and Blood Pesticide Levels' study, which was conducted with the decision of the ethics committee dated 08.01.2021 and numbered 2021/81. The blood samples were collected from female patients who signed informed consent forms and consented to the research using genetic material for identification. Patient names were anonymized in the study. We measured ssDNA levels in the selected blood samples using the Qubit ssDNA Assay Kit (Life Technologies, Carlsbad, CA, USA).

DNA quantification

DNA isolation

We added 15 µl Proteinase K to 2 ml Eppendorf tubes for DNA isolation from blood. We added 250 µl well-vortexed blood and 300 µl Extraction Buffer. We vortexed for 10–15 seconds. We incubated in a water bath at 60°C for 20 minutes, then vortexed again for 10–15 seconds. We added 300 µl Binding Buffer and vortexed for 10–15 seconds. We centrifuged the samples for 1 minute at 10,000 rpm in 2 passes. 650 µl Wash Buffer 1 was added to the spin column and centrifuged for 1 minute at 10,000 rpm. 500 µl Wash Buffer 2 was added to the spin column and centrifuged for 1 minute at 10,000 rpm. 250 µl Wash Buffer 1 was added to the spin column and centrifuged for 3 minutes at 12. Then, we placed the spin columns into 1.5 ml Eppendorf, where the DNA will be stored. The spin column filter was aligned, and 50 µl of Elution Buffer was added. The tubes were capped and left at room temperature for 5 minutes, then centrifuged at 10,000 rpm for 1 minute.

DNA measurement protocol

Qubit ssDNA Test (Life Technologies, Carlsbad, CA, USA) Kit components were brought to room temperature. The ssDNA Reagent was diluted 1:200 with ssDNA Buffer. 190 µl of the working solution was added to each sample tube. 10 µl of DNA from each sample was added. Light vortexing was performed for 2-3 seconds. Incubation was performed for 2 minutes at room temperature and in the dark, and the measurement results were recorded.

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Whole exome sequencing analysis (WES analysis) method

Bioinformatics analysis was performed by a research laboratory specialized in the relevant analysis field. "Whole Exome Sequencing" was performed using the Next Generation Sequencing method. All detected variants (pathogenic, likely pathogenic, and VUS) were evaluated with Genemaster, ClinVar, VarSome, and "in-slico" prediction programs based on the clinical information of the patient. ACMG (American College of Medical Genetics) criteria were applied for variant classification.

Statistical method

Statistical Package for the Social Sciences (SPSS, version 20.0; IBM Corp. 2019 IBM SPSS Armonk, NY, version 20.0. Armonk, NY, IBM Corp.) was used for data analysis. The data obtained were presented with descriptive statistics (arithmetic mean, median, standard deviation, and percentage distributions). When comparing the means between groups, Kolmogorov-Smirnov and Shapiro-Wilk tests evaluated the conformity to normal distribution. We used the Mann-Whitney U test to determine the differences in ssDNA concentrations of categorical variables. The chi-square test was used to compare the percentage distributions of categorical data between groups, and the Spearman-Ra test was used to evaluate the correlation of two continuous data between groups, and the bivariate correlation test was used to evaluate the correlation of two continuous data between groups. The odds ratio and Risk were measured between groups deemed to be related.

Ethical approval

Ethics committee approval of this study was obtained in the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee meeting resolutions dated 07.10.2022 and numbered 2022/499.

Results

We included 91 patients in the study. There were 56 (61.5%) women in the group with pesticide exposure and pregnancy loss, and 35 (38%) women in the group that had no pesticide exposure and had normal delivery. We have shown the demographic data and variables obtained within the scope of the study in Table 2. In the comparisons between the groups, the history of contact with agricultural products was significantly higher in the pesticide contact group ($p<0.05$). There was no significant relationship between other demographic characteristics. 8.8% of women in the study had three or more miscarriages (habitual abortion). All of the women who experienced habitual abortion had pesticide exposure ($p<0.05$).

Table 2. Relationship between groups and variables

Variables	Groups		p value
	Pesticide positive n=56	Pesticide negative n=35	
Age			
mean (±SD)	28.4 (±4.38)	28.4± (4.88)	0.592
min-max	21-35	19-35	
Educational status n (/%)			
Litarete	1 (1.8%)	2 (5.7%)	
Primary scholl graduate	24 (42.9%)	13 (37.1%)	0.191*
Secondary-high school graduate	29 (51.9%)	15 (42.9%)	
Undergraduate education and above	2 (3.6%)	5 (14.3%)	
Pesticite exposure history	18 (32.1%)	1 (2.9%)	**0.001***
Body mass index			
mean (±SD)	26± (2.52)	27.1± (3.2)	0.124
min-max	20.2-32.8	20.2-40.4	
Number of pesticite			
mean (±SD)	5.2±1.83	0	
min-max	(2-11)		
Total number of pregnancy			
mean (±SD)	3.07 (±1.12)	2.71 (±1.20)	0.145*
min-max	1-5	1-5	
Total number of abortions			
mean (±SD)	1.68 (±0.765)	0.54 (±0.611)	**0.000**
min-max	1-4	0-2	
History of abortion	29 (51.8%)	17 (48.6%)	0.765*
Total of normal births			
mean (±SD)	1.77 (±1.07)	1.66 (±0.83)	0.621
min-max	0-4	0-4	
SsDNA level (ng/µL)			
mean (±SD)	190.94 (±97.05)	213.5 (±159)	0.984
min-max	43.40-463.80	27-712	

p value: Mann Whitney U Test *:p value chi-square

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Statistically, there was no statistical relationship between ssDNA ratios and the groups (p>0.05). The level of ssDNA in the blood of women who had miscarriages was significantly higher than in women who had never had miscarriages (p<0.05) Table 3.

Table3. Association between ssDNA level and abortion history

	n(%)	ssDNA level (ng/µL) n(SD) min-max	Mean rank	Z	p value
No abortion	18	152.7±111.4 (27-478.7)	33.94	-2.162	0.031
At least one abortion	73	211.1±125 (43-712)	48.97		

p value: Mann Whitney U Test

The pesticides that were detected to be present at high levels in the previous study and found to be present in the patient group included in this study are as follows. β-Hexachlorocyclohexane (β-HCH), delta-hexachlorocyclohexane (δ HCH), Hexachlorobenzene (HCB), Pentachlorophenol-28 (PCP-28), Pentachlorophenol-52 (PCP52), o,p'-Dichlorodiphenyldichloroethylene (o,p'-DDE), p,p'- Dichlorodiphenyldichloroethylene (p,p'-DDE), o,p'- dichlorodiphenyldichloroethane (o,p'-DDD), p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), Pentachlorobiphenyl-118 (PCB-118), Pentachlorobiphenyl-101 (PCB-101), Pentachlorobiphenyl-153 (PCB-153), Pentachlorobiphenyl-138 (PCB-138), Pentachlorobiphenyl-202 (PCB-202), Pentachlorobiphenyl-180 (PCB-180), Acetamibride, Thiomethane, Thiomedoxane.

When ssDNA levels of these pesticides were compared, a significant correlation was found between PCB52 and ssDNA (p<0.05). Table 4. The odds ratio is 0.312 (95% confidence interval 0.107–0.905) for PCB 52. When the relationship between the measured amount of organochlorine and organophosphate pesticides and ssDNA level was screened by correlation analyses, the comparison results were not significant (p>0.05) o,p'-DDE (p=0.046), PCB-52 (P=0.002), and PCB-153 (p=0.020) were higher in multiparous women compared to primiparous women. Multiparous women were significantly heavier than primiparous women (p<0.05). 21.1% of the participants reported having contact with an agricultural product at some point. The relationship between pesticide exposure and the participants' length of residence, the distance between agricultural land and residence, and consumption habits of red meat, poultry, fish, milk, fresh fruits and vegetables, and eggs could not be determined from these data (p>0.05).

Mutations that may be responsible for the etiology of miscarriage were detected in two of the 4 NGS analyses. In the Whole Exome Sequence analysis of the patient, the *DYNC2H1* gene NM_001377.3 c.7506G>A (p.Trp2502Ter) variation was heterozygous. The DYNC2H1 gene NM_001377.3 c.8458C>A (p.Pro2820Thr) variation was heterozygous in another patient's Whole Exome Sequence analysis.

Table 4. Relationship between pesticides and ssDNA levels

Pesticides		n (%)	ssDNA levels (ng/µL) Mean±SD	p value*
PCB-180	**N**	51	208.03±143	0.801
	Y	40 (44%)	188.09±95	
PCB-202	**N**	72	202.6±176	0.796
	Y	19 (20.3%)	188.0±102	
PCB-138	**N**	66	197.0±132	0.319
	Y	25 (27.5%)	206.5±104	
***p.p'*-DDT**	**N**	78	197.9±125	0.687
	Y	13 (14.3%)	209.9±131	
***o.p'*-DDT**	**N**	89	197.1±124	0.117
	Y	2 (2.2%)	310.5±99	
PCB-153	**N**	85	196.7±125	0.227
	Y	6 (6.6%)	240.6±105	
PCB-118	**N**	83	200.7±127	0.989
	Y	8 (8.8%)	187.9±97	
***p.p'*-DDD**	**N**	70	203.6±131	0.903
	Y	21 (23.1%)	186.2±101	
***o.p'*-DDD**	**N**	70	202.8±130	0.985
	Y	20 (22%)	188.2±103	
PCB-101	**N**	79	200.5±129	0.944
	Y	12 (13.2%)	193.5±91	
***p.p'*-DDE**	**N**	88	201.4±126	0.504
	Y	3 (3.3%)	145.8±53	
***o.p'*-DDE**	**N**	76	208.4±130	0.103
	Y	15 (16.5%)	154.8±76	
PCB-52	**N**	73	190.9±127	0.045
	Y	18 (19.8%)	238.4±108	
PCB-28	**N**	46	209.8±149	0.990
	Y	45 (49.5%)	189.1±93	
GamaHCH	**N**	62	209.1±137	0.480
	Y	29 (31.9%)	179.2±90	
Delta HCH	**N**	86	201.0±127	0.972
	Y	5 (5.5%)	175.2±45	
BetaHCH	**N**	87	200.3±126	0.889
	Y	4 (4.4%)	183.1±61	
Acetamibrid	**N**	88	210.7±125	0.362
	Y	3 (3.3%)	136.3±107	
Thiometan	**N**	90	201.2±124	0.132
	Y	1 (1.1%)	56.7	
Thiomedoxan	**N**	90	201.7±125	0.198
	Y	1 (1.1%)	70.4	

*Mann Whitney U p value, SD: standart deviation

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Discussion

One of the study's main results is the correlation between ssDNA levels and PCB-52, one of the polychlorinated biphenyls, and the finding that ssDNA levels are higher in women who have had spontaneous abortions than in those who have not.

Pesticides used in the fight against weeds and crop pests spread to the ecosystem through air, water, and soil and accumulate in living organisms. Pesticides' lipophilic structure and stability properties cause them to accumulate in the maternal adipose tissue and pass through the placenta to the fetus. Pesticides trigger oxidative stress, damaging DNA by producing reactive oxygen species [8]. DNA damage can result in breakage in a single DNA strand or alter the DNA double helix. The type of these damages will vary depending on the cell's age, type, and environmental circumstances. DNA breaks that exceed repair mechanisms are associated with diseases [9]. Genotoxic, mutagenic, teratogenic and endocrine disrupting effects of pesticides have been demonstrated by many studies [10]. Exposure to these toxic agents in early pregnancy has been associated with many effects, including intrauterine growth retardation, fetal and postnatal death, congenital disabilities, preterm delivery, impaired cognitive development, and immunological dysfunction [11,12]. Effects on female reproductive health have been associated with menstrual disorders, early menopause, delayed menarche, ovarian dysfunction, reduced fertility, and adverse pregnancy outcomes [13].

In this study, we examined the effect of pesticides on DNA in a patient population in which the inclusion criteria were very narrowly defined to reveal the genotoxic effect of environmental factors. There was no significant correlation between pesticide amounts and ssDNA levels, and ssDNA levels were significantly higher only in cases in which PCB 52 was detected in the blood. The chemical name of PCB 52 is 2,22,5,52-tetrachlorobiphenyl. PCB52, one of 209 types of PCB compounds, is known to cause developmental and physiological anomalies in the reproductive system due to both structural and estrogenic effects [14,15]. Sandal et al. demonstrated the genotoxic effects of PCB 52 in human peripheral lymphocyte cultures. [16]. A study conducted in rats showed that PCB 52 in the gestational period is associated with neurodevelopmental disorders in offspring and hepatotoxicity [17]. Studies show that single-chain DNA breaks are associated with mutagenicity and carcinogenicity in PCB-exposed hepatocytes [18,19]. In this respect, we think it is essential to reveal the relationship between ssDNA and PCB-52 in spontaneous abortion cases. The fact that PCBs used in electrical insulation, paints, plastics, and adhesives are still detected even though their production was completely stopped in 1977 and their use was banned in many countries is also an example of the long-term effects of pesticides in terms of human health and the environment [20]. The detection of PCBs and organochlorine pesticides in breast milk in a study conducted in Turkey in 2021 shows that the problem continues. In this study by Agus et al., although the amount of pesticide was found to be slightly less in multiparous women, in this study, when the value in the blood was examined, *op-'* DDE, PCB-52, and PCP-153 were found to be significantly higher in multiparas [21,22]. Multiparous pregnant women may be due to different characteristics between the groups included in the study, such as being overweight compared to primiparas in this study.

The relationship between pesticides and reproductive toxicity is essential in revealing environmental factors' effect. Pandey et al. found that organochlorine and organophosphate compounds were significantly higher in cases of recurrent spontaneous abortion [23]. In recurrent miscarriages, tests are performed to identify and treat causes that may shed light on the current etiology. For example, thrombophilias metabolic and endocrine disorders of maternal origin are some treatable causes. Some scoring systems based on independent risk factors are being developed to predict these recurrent cases, which can be physically and psychologically devastating for a woman [24]. There is a need for more studies in which cause and effect relationships will be revealed for ssDNA ratio or pesticides to be a biomarker to elucidate the etiology in the clinical process. As a result of this study, we found that ssDNA levels were higher in women with a history of miscarriage. The fact that there was a significant correlation between cases with no previous miscarriage and cases with at least one previous miscarriage suggests that this issue can be evaluated. There are studies on the predictive value of ssDNA elevation in patients with hepatocellular carcinoma [6].

There are also publications in the literature about mutation in the *DYNC2H1* gene and recurrent miscarriage [25,26]. In this study, mutations were detected in two samples. In the Whole Exome Sequence analysis of Case 1, the variation of *DYNC2H1* gene NM_001377.3 c.7506G>A (p.Trp2502Ter) was found to be heterozygous. This variation is considered Likely Pathogenic in the VarSome annotation database [27]. *DYNC2H1* gene mutations are associated with the clinic *"Short-rib thoracic dysplasia 3 with or without polydactyly, [OMIM:613091]"* in the OMIM database, but these gene mutations are also associated with the clinic *"Pregnancy loss, recurrent, early?"* in the HGMD 2021.4 professional database [28]. In the Whole Exome Sequence analysis of Case 2, the *DYNC2H1* gene NM_001377.3 c.8458C>A (p.Pro2820Thr) variation was heterozygous. This variation is considered VUS (Variant of Unknown Clinical Significance) in the VarSome annotation database. In the OMIM database, DYNC2H1 gene mutations are associated with the clinic *"Short-rib thoracic dysplasia 3 with or without polydactyly, [OMIM:613091]",* but these gene mutations are also in the HGMD 2021.4 professional database *"Pregnancy loss, recurrent, early?"* associated with the clinic. In the other two analyses, no variation was detected in the Whole Exome Sequencing Analysis that could be related to the patient's clinic [29]. The discovery of this mutation in this study is valuable in contributing to the literature. Both of these cases were selected because they had higher-than-average pesticide residues. Both cases had repeated abortions and had high levels of PCP and organochlorine pesticides such as *p,p′*-DDT, *p,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, and *p,p′*- DDE. Which pesticide or pesticides have a primary mutagenic effect should be further investigated in larger groups. These data support the studies on the relationship

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between genotoxic and mutagenic properties of DDT exposure [30].

In the US Collaborative Perinatal Project (CPP), increased DDE concentrations were associated with an increased risk of fetal loss in previous pregnancies, with a median serum DDE level of 24.5 µg/L in mothers who experienced abortion. Although median values vary in different populations due to differences in environmental factors, studies have highlighted increased risks against increasing rates [31]. Although there are banned products in the community, the fact that exposures have been determined shows that conscious practices and control mechanisms should be activated more strictly. Research on the relationship between genotoxicity and pesticides should be conducted in larger study populations. Studies that reveal the effect of persistent pollutants in pregnancy losses whose etiology cannot be elucidated will also guide health authorities in organizing global health policies.

The limitations of this study are that it was conducted with a limited number of patients, the malefactor was not evaluated in cases of miscarriage, and laboratory data did not support it.

Conclusion

Studies in this area need to be increased in order to determine the relationship of ssDNA elevation with pesticide exposure, its predictive value in terms of abortion and the value of environmental factors in repeated abortions whose etiology has not been clarified.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

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Ethical Approval

Ethics committee approval of this study was obtained in the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee meeting resolutions dated 07.10.2022 and numbered 2022/499.

References

1. Turesheva A, Aimagambetova G, Ukybassova T, et al. Recurrent pregnancy loss etiology, risk factors, diagnosis, and management. Fresh look into a full box. J Clin Med. 2023;12:4074.
2. El Hachem H, Crepaux V, May-Panloup P, et al. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017;9:331-45.
3. Settimi L, Spinelli A, Lauria L, et al. Spontaneous abortion and maternal work in greenhouses. Am J Ind Med. 2008;51:290-5.
4. Han X, Lu T, Hu Y, et al. A metabolomic study on the effect of prenatal exposure to Benzophenone-3 on spontaneous fetal loss in mice. Ecotoxicol Environ Saf. 2022;233:113347.
5. Venners SA, Korrick S, Xu X, et al. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. Am J Epidemiol. 2005;162:709-16.
6. Zhao Q, Xu Y, Yuan D, et al. Role of ssDNA as a noninvasive indicator for the diagnosis and prognosis of hepatocellular carcinoma: an exploratory study. Dis Markers. 2021;2021:9958909.
7. Erdfelder E, Faul F, Buchner A. GPOWER: A general power analysis program. Behavior Research Methods, Instruments, & Computers. 1996;28:1-11.
8. Kumar S, Sharma A, Kshetrimayum C. Environmental & occupational exposure & female reproductive dysfunction. Indian J Med Res. 2019;150:532-45.
9. Mozaffarieh M, Schoetzau A, Sauter M, et al. Comet assay analysis of single-stranded DNA breaks in circulating leukocytes of glaucoma patients. Mol Vis. 2008;14:1584-8.
10. Zúñiga-Venegas LA, Hyland C, Muñoz-Quezada MT, et al. Health effects of pesticide exposure in latin american and the caribbean populations: a scoping review. Environ Health Perspect. 2022;130:96002.
11. Pascale A, Laborde A. Impact of pesticide exposure in childhood. Rev Environ Health. 2020;35:221-7.
12. La X, Wang W, Zhang M, Liang L. Definition and multiple factors of recurrent spontaneous abortion. Adv Exp Med Biol. 2021;1300:231-57.
13. Wang Q, Wei L-W, Zhou W-T, et al. PCB28 and PCB52 induce hepatotoxicity by impairing the autophagic flux and stimulating cell apoptosis in vitro. Toxicol Lett. 2018;289:28-41.
14. Zhang Y, Jin X, Han H, Li Z. Effects of 2,2',5,5'-tetrachlorobiphenyl (PCB52) on migration of chicken primordial germ cells. Reprod Fertil Dev. 2005;17:587-91.
15. Vincent DR, Bradshaw WS, Booth GM, et al. Effect of PCB and DES on rat monoamine oxidase, acetylcholinesterase, testosterone, and estradiol ontogeny. Bulletin of Environmental Contamination and Toxicology. 1992;48:884-893.
16. Sandal S, Yilmaz B, Carpenter DO. Genotoxic effects of PCB 52 and PCB 77 on cultured human peripheral lymphocytes. Mutat Res. 2008;654:88-92.
17. Xu LL, Zhang QY, Chen YK, et al. Gestational PCB52 exposure induces hepatotoxicity and intestinal injury by activating inflammation in dam and offspring mice: a maternal and progeny study. Environ Pollut. 2022;313:120186.
18. Sina JF, Bean CL, Dysart GR, et al. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res. 1983;113:357-91.
19. Appelgren H, Hedenskog M, Sandström C, et al. Polychlorinated biphenyls induce meiotic length mutations at the human minisatellite MS32 in yeast. Environ Mol Mutagen. 1999;34:285-90.
20. Ross G. The public health implications of polychlorinated biphenyls (PCBs) in the environment. Ecotoxicol Environ Saf. 2004;59:275-91.
21. Grimm FA, Hu D, Kania-Korwel I, et al. Metabolism and metabolites of polychlorinated biphenyls. Crit Rev Toxicol. 2015;45:245-72.
22. Agus S, Akkaya H, Daglioglu N, et al. Polychlorinated biphenyls and organochlorine pesticides in breast milk samples and their correlation with dietary and reproductive factors in lactating mothers in Istanbul. Environ Sci Pollut Res Int. 2022;29:3463-73.
23. Pandey A, Jaiswar SP, Ansari NG, et al. Pesticide risk and recurrent pregnancy loss in females of subhumid region of India. Niger Med J. 2020;61:55-9.
24. Dai YF, Lin LZ, Lin N, et al. APA scoring system: a novel predictive model based on risk factors of pregnancy loss for recurrent spontaneous abortion patients. J Obstet Gynaecol. 2022;42:2069-74.
25. Qiao Y, Wen J, Tang F, et al. Whole exome sequencing in recurrent early pregnancy loss. Mol Hum Reprod. 2016;22:364-72.

DOI: 10.5455/medscience.2023.09.190

26. Hassan E, Kojima R, Ozawa F, et al. Abnormal ciliogenesis in decidual stromal cells in recurrent miscarriage. J Reprod Immunol. 2022;150:103486.

27. VARSOME. https://varsome.com/ access date 01.07.2023.

28. OMIM. https://www.omim.org/ access date 01.08.2023.

29. Rajcan-Separovic E. Next generation sequencing in recurrent pregnancy loss-approaches and outcomes. Eur J Med Genet. 2020;63:103644.

30. Petrelli G, Figà-Talamanca I, Lauria L, Mantovani A. Spontaneous abortion in spouses of greenhouse workers exposed to pesticides. Environ Health Prev Med. 2003;8:77-81.

31. Eskenazi B, Chevrier J, Rosas LG, et al. The Pine River statement: human health consequences of DDT use. Environ Health Perspect. 2009;117:1359-67.