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Field Study

Occupational Exposure to Organophosphate and Carbamate Pesticides Affects Sperm Chromatin Integrity and Reproductive Hormone Levels among Venezuelan Farm Workers

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Abstract: Occupational Exposure to Organophosphate and Carbamate Pesticides Affects Sperm Chromatin Integrity and Reproductive Hormone Levels among Venezuelan Farm Workers: Leticia Miranda-Contreras, *et al.* Electron Microscopy Center "Dr. Ernesto Palacios Prü", University of Los Andes, Venezuela—Objectives: Several reports suggest that chronic pesticide exposure may affect semen quality and male fertility in humans. The objective of this study was to evaluate the association between occupational exposure to organophosphate (OP) and carbamate (CB) pesticides and semen quality, as well as levels of reproductive and thyroid hormones of Venezuelan farm workers. **Methods:** Thirty-five healthy men (unexposed group) and 64 male agricultural workers (exposed group) were recruited for clinical evaluation of fertility status. Fresh semen samples were evaluated for sperm quality and analyzed for DNA fragmentation index (DFI) by flow cytometry. Pesticide exposure was assessed by measuring erythrocyte acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BuChE) with a Test-mate ChE field kit. Serum levels of total testosterone (Tt), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid stimulating hormone (TSH) and free thyroxine (FT4) were analyzed using enzyme immunoassay kits. **Results:** Evidence of pesticide exposure was found in 87.5% of farmers based on AChE and BuChE inhibition. Significant increments were observed in sperm DFI with significant decreases in some semen parameters. DFI was negatively correlated with BuChE, sperm concentration, morphology and vitality in these workers. The levels of Tt, PRL, FT4 and TSH appeared to be normal; however, there was a tendency for increased LH and FSH levels in exposed workers. **Conclusions:** Our results confirm the potential impact of chronic occupational exposure to OP/CB pesticides on male reproductive function, which may cause damage to sperm chromatin, decrease semen quality and produce alterations in reproductive hormones, leading to adverse reproductive health outcomes.
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Key words: Acetylcholinesterase, Butyrylcholinesterase, Luteinizing hormone, Organophosphate/carbamate pesticides, Sperm chromatin, Sperm quality

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Venezuelan farm workers are exposed daily to OP and CB pesticides which are widely applied in agriculture as insecticides, herbicides or fungicides. The neurotoxic effects of OP and CB pesticides have been well documented. Moreover, several studies have associated short- and long-term exposures to these pesticides with altered endocrine function, which may result in adverse health effects on fetal/child development, metabolism, reproductive function and cancer[1–4]. These toxic compounds are potent inhibitors of the enzyme AChE, located in nerve terminals and erythrocytes, as well as BuChE that is present in plasma. Measures of cholinesterase inhibition have been well accepted as biomarkers of adverse neurotoxic effects

in humans caused by exposures to OP and CB pesticides[5–8].

Several epidemiological studies have demonstrated an association between pesticide exposure and altered semen quality, which directly or indirectly affects fertility and reproduction in agricultural workers[9–12]. Most of the data are from occupational studies linking exposure to various organochlorine and OP pesticides with sperm DNA damage and oxidative stress[3, 13–17]. These pesticides have been considered to be environmental endocrine-disrupting chemicals (EDCs), which are compounds that alter the normal functioning of the endocrine system of both wildlife and humans[18]. EDCs act mainly by interfering with natural hormones because of their strong potential to bind to various estrogen or androgen receptors. EDCs may also interfere with the synthesis, transport, metabolism and elimination of hormones, thereby altering the concentration of natural hormones. An increase in the number of human male reproductive developmental disorders, such as cryptorchidism, hypospadias, testicular cancer and decreased quality of semen, have been proposed to be due to the action of EDCs[19]. OP pesticides are suspected to alter reproductive function by reducing brain AChE activity and secondarily influencing the gonads. In addition, OP and CB pesticides have been shown to disrupt the thyroid system which is essential for normal brain development, for the control of metabolism and for many aspects of normal organ functions[18, 20].

Despite the known toxic effects of agropesticides which impact human endocrine and reproductive functions, agricultural workers in the developing world are not provided at all with occupational health and safety care benefits. It is essential to monitor the exposure and health of farm workers and to impart them with the knowledge to prevent potentially dangerous levels of exposure to pesticides. The objective of this study was to evaluate the association between occupational exposure to OP/CB pesticides and semen quality, as well as reproductive and thyroid hormone profiles of Venezuelan farm workers.

Materials and Methods

Study area

We conducted a cross-sectional study in an agricultural community of the Municipality of Rivas Dávila, Mérida State, Venezuela, during 2009–2010. This region is one of the principal agricultural production areas in the southwestern part of Venezuela, whose main products are vegetables (cabbage, lettuce, peppers, onions, garlic, celery and tomatoes), tubers (potatoes, carrots), fruits (strawberry, passionflower, figs) and flowers (roses, astromelias, lilies, gerberas). It is situated at 1,800 m above sea level, and because of its temperate climate, agricultural production is possible all year round. The most frequently applied pesticides include OPs, CBs, dithiocarbamates, pyrethroids and triazines, among others.

Study population

We randomly selected 64 male farm workers, who were residents of this community for at least two years prior to the study. Inclusion criteria for subjects in the exposed group included history of work with pesticides and no history of hepatic, renal and metabolic diseases, as well as pathologies that affect male reproductive function, such as orchitis, varicocele, cryptorchidism, or recently acquired sexually transmitted disease. Subjects in the control group consisted of 35 healthy men, who were not currently exposed to pesticides, either occupationally or non-occupationally; they were randomly recruited in Mérida City, which is about 90 km from the agricultural region of Rivas Dávila.

The ages of the subjects ranged from 18 to 52 years old. Each individual was interviewed directly regarding his sociodemographic characteristics, occupational activities, alcohol consumption, smoking habits and clinical characteristics. Each participant signed an informed consent form and donated blood and semen samples. The study protocol was approved by the Ethics Committee of the Council of Scientific, Humanistic, Technologic and Artistic Development of the University of Los Andes, Mérida State, Venezuela.

Semen collection and analysis

All participating subjects were asked to abstain from ejaculation and drinking alcohol for 3–7 days before semen collection. Semen was collected in a sterile 100-m*l* plastic container by masturbation and analyzed on-site within one hour for both macroscopic and microscopic characteristics. Two studies were applied to each semen sample: 1) Conventional physical and cytomorphological characteristics of fresh semen samples were evaluated according to World Health Organization (WHO) guidelines[21]. 2) The integrity of sperm DNA was analyzed with ethanol-fixed specimens by flow cytometry using the Sperm Chromatin Structure Assay[22].

Semen analysis included: liquefaction time, seminal volume, pH, sperm concentration, total sperm number, sperm morphology, sperm motility, sperm viability and leukocyte concentration to rule out leukocytospermia. A Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) was used to determine sperm and leukocyte concentrations.

To determine sperm concentration and motility, samples were incubated at 37°C for 20 minutes to liquefy them. Five microliters of each sample was

placed in the Makler chamber and by looking at the sample under a microscope, the examiner counted the number of spermatozoa in a defined field of view. The procedure was repeated to ensure accuracy and a conversion factor was used to calculate the concentration. The total number of sperms was obtained by multiplying the sperm concentration by the volume of ejaculate and the value was expressed in millions of spermatozoa per ejaculation. Sperm motility was also analyzed microscopically. The number of motile sperms, viewed in 5 distinct fields of each sample, was counted until a total of 200 spermatozoa were assessed. Sperm morphology was assessed microscopically using a smear of fresh semen sample that was stained with Diff-Quick in order to view and count the number of normal and abnormal spermatozoa until at least 200 consecutive spermatozoa were evaluated. Both sperm motility and morphology evaluations were done according to WHO criteria[21].

Sperm vitality was assessed using an eosin dye. A total of 200 spermatozoa were analyzed under a phase contrast light microscope (400X) to distinguish the live (unstained) sperm from the dead (stained) sperm, and the value was expressed as the percentage of viable sperm.

Leukocytes in semen were measured with peroxidase stain using ortho-toluidine blue, as suggested by the WHO[21]. The percentage of peroxidase-positive neutrophiles was recorded. Leukocytospermia is diagnosed if a semen sample has more than 1 million peroxidase-positive leukocytes[21].

Sperm Chromatin Structure Assay (SCSA)

The rate of DNA fragmentation was determined by employing the SCSA method based on the procedure described by Evenson and Jost[23] but with slight modifications[24]. Briefly, an aliquot of fresh sperm sample was washed twice with TNE buffer (0.01 M Tris HCl, 0.15 M NaCl, 1 mM EDTA, pH 7.4) and centrifuged, and the sediment was fixed in 70% ethanol. After washing three times by centrifugation with TNE buffer, the sample was treated with a denaturizing acidic solution (0.1% Triton X100, 0.15 M NaCl, 0.08 N HCl, pH 1.4) for 30 seconds and stained with acridine orange. The sample was then analyzed in a flow cytometer (FACSort, Cell Quest software, Becton Dickinson, San Jose, CA, USA). Due to the metachromatic properties of acridine orange, it is possible to distinguish between double-stranded (ds) DNA (green fluorescence) and single-stranded (ss) DNA (red fluorescence) when exposed to 488 nm laser light from a flow cytometer.

The SCSA parameters obtained from the measurement included the DNA fragmentation index (DFI), which is the ratio of ss DNA over the sum of ds DNA and ss DNA. The results of the DFI were expressed as percentages and considered normal when they were below 30%[25, 26].

Blood collection and hormone analysis

Venous blood samples were collected in heparinized tubes after a 12-h overnight fast and then plasma was separated by centrifugation for hematological and biochemical tests. Serum was collected and kept frozen until assayed for reproductive and thyroid hormones. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (TSH) and free thyroxine (FT4) were measured by enzymatic immunoassay using ELISA kits (Right Choice Diagnostics, Muenster, Germany) and serum testosterone level was analyzed using an ELISA kit (DRG Instruments GmbH, Marburg, Germany).

Analysis of cholinesterase activities: erythrocyte acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BuChE)

A capillary blood sample drawn from the farmer's fingertip was used to test the levels of AChE in erythrocytes and BuChE in plasma, using a portable field kit (Test-mate EQM Research, Cincinnati, OH, USA), based on the method of Ellman *et al.*[27]. Acetylthiocholine was the substrate for AChE, and the thiocholine produced was coupled with dithiobis nitrobenzoic acid, which was quantified spectrophotometrically at 450 nm. AChE activity was expressed as μmol/min/gHb (U/m*l*). In the same manner, plasma BuChE activity was measured from the hydrolysis of butyrylcholine iodide, and data were expressed as μmol/min/m*l* plasma (U/m*l*). Evaluation of Test-mate ChE results were based on AChE and BuChE inhibition associated with different degrees of clinical severity, according to Rajapakse *et al.*[28], and were as follows: "normal" ≥75%, "mild inhibition" 30% to 74%, "moderate inhibition" 10% to 29%, and "severe inhibition" <10%. Acute and chronic exposures were defined as low acute (inhibition of AChE or BuChE >25%), high acute (inhibition of AChE >30% or BuChE >40%) and chronic (inhibition of AChE in erythrocytes >60%)[5].

Statistical analysis

Continuous variables were expressed as means ± standard deviation. To establish differences between continuous variables, we used analysis of variance (ANOVA) to compare age groups. The Student's *t*-test was applied to detect differences between the means of two normally distributed populations. Bonferroni correction was employed to account for multiple comparisons. The degree of association

between variables was evaluated based on Pearson's or Spearman's correlation coefficient. Statistical analysis was performed with SPSS version 15 for Windows (SPSS Inc., Chicago, IL, USA). Two-sided p values of <0.05 were considered statistically significant.

Results

The characteristics and results of biochemical analysis of the study population are presented in Table 1. The mean age of the studied farm workers was 33.5 years, and their ages ranged from 18 to 52 years. The age group containing the most participants was that containing farm workers who were 18–28 years old (37%), followed by those 29–39 years old (33%) and finally the group older than 40 years old (30%). According to the period of exposure to pesticides, 53.5% had worked in agriculture for more than 5 years, 22.5%, for 2–5 years and 11.3%, for less than 2 years. Seventy five percent of the workers reported spraying pesticides from one to three times a week, 8% sprayed once or twice a month and 3% applied pesticides daily.

The mean (± SD) age of the control group was 25.1 ± 5.5 years. About the same proportion of participants in both control and exposed groups reported alcohol (72.7% and 66.2%, respectively) or tobacco (24.2% and 29.6%, respectively) consumption. Various biochemical parameters in both groups were within the reference values, and the majority showed no significant difference except for the results of total cholesterol, with mean values of 202.4 ± 39.5 and 161.1 ± 36.0 mg/d*l* in the exposed and control groups, respectively (p<0.0001); however, these results were still within normal limits.

Table 2 presents semen characteristics of the study population. Significant differences in some seminal characteristics, such as higher seminal pH (p=0.004) and lower percent live sperm (p<0.0001), were observed in the exposed group as compared with that of the control group; on the other hand, a marginally significant difference was seen for rapid and progressive sperm motility (p=0.05).

Table 3 shows the distribution of AChE and BuChE results in the studied agricultural workers according to clinical category of enzyme inhibition, based on the study of Rajapakse *et al*.[28]. Overall, the participants in this study showed more depressed BuChE activity compared with erythrocyte AChE activity.

The levels of BuChE activity indicated that 87.5% of the farm workers had been exposed to OP and CB pesticides; 82.8% (N=53) had mild inhibition, with their mean BuChE activity (1.5 ± 0.3 U/m*l*) decreased by 31.6% from normal values (2.2 ± 0.2 U/m*l*). On the other hand, 35.9% (N=23) of the farm workers showed mild inhibition of AChE activity (3.1 ± 0.4 U/m*l*), which was reduced by 24.4% from normal values (4.1 ± 0.4). Two study participants had moderate cholinesterase inhibition, one showing a reduction of BuChE activity of 69.3% and the other, a depression of AChE activity of 73.1%, as compared with normal values.

Table 4 shows the levels of AChE and BuChE activities, SCSA results, expressed as the DFI, and some semen quality parameters, concentration, morphology and percent live sperm, in the exposed study population according to age groups: 18–28 years, 29–39 years and ≥40 years. To account for the increased possibility of a type I error due to multiple simultaneous hypothesis tests, we applied a Bonferroni correction based on two outcome measures and seven predictors (age considered as a single predictor), where α=0.05/14=0.00357. Significant decreases in BuChE (p=0.003) activities were found in all age groups compared with the control group. However, sperm DFI values were significantly increased (p<0.0001) in the exposed workers as compared with the control subjects. After the Bonferroni adjustment, the differences in AChE activity were not significant. On the other hand, semen parameters of exposed workers showed significant decrements in sperm concentration (p=0.002) and percent live sperm (p<0.0001) but not a significant decrease in normal morphology (p=0.05) compared with control values. The poorest semen quality parameters were seen among the group ≥40 years old, showing lower sperm concentrations compared with the 18–28-year-old group and decreased percentages of morphologically normal sperm and live sperm compared with the group of 29–39-year-old group.

Figure 1 shows the correlation analysis between sperm DFI and plasma BuChE activity, sperm concentration, morphology and vitality in the exposed workers. BuChE activity was negatively correlated with sperm DFI in exposed workers (Fig. 1a); although there was only a small correlation between both variables (r=–0.291), the abnormal levels of BuChE activity were significantly associated with abnormal values of DFI (p=0.027). There is a possibility that both variables are independently responding to a third factor, such as chronic pesticide exposure, and that the BuChE activity does not causally affect the sperm DFI. On the other hand, these studies revealed a strong negative correlation between sperm DFI and concentration (r=–0.522, p=0.0004, Fig. 1b), and medium correlation between sperm DFI and morphology (r=–0.427, p=0.001, Fig. 1c) as well as vitality (r=–0.445, p=0.001, Fig. 1d), suggesting that the workers were experiencing pesticide exposure.

Table 5 shows the distribution of reproductive (FSH, LH, PRL, Tt) and thyroid (TSH, FT4) hormone

Table 1. Characteristics and results of biochemical analysis of the study population

Variable	Exposed group (N=64)	Control group (N=35)	Reference value
Age	34 ± 10 (18–52)	25 ± 6 (18–42)	—
Alcohol consumption	66.2%	72.7%	—
Smoking	29.6%	24.2%	—
Years exposed: >5 years	53.5%	0	—
2–5 years	22.5%	0	
<2 years	11.3%	0	
Frequency of fumigation: Daily	3%	0	—
1x–3x / week	75%	0	
1x–2x / month	8%	0	
Body mass index (BMI) (kg/m^2)	20.2 ± 3.3 (14.4–29.9)	24.2 ± 3.6 (18.5–31.7)	19–25
Hemoglobin (Hb) (g/d*l*)	15.4 ± 0.8 (13.6–17.2)	15.6 ± 1.0 (13.4–17.3)	12–18
Hematocrit (g/d*l*)	47.8 ± 2.1 (44–52.6)	47.5 ± 3.1 (41.3–52.3)	37–51
White blood cells (WBC) (K/μl)	6.3 ± 1.4 (4.2–9.7)	5.7 ± 1.0 (4.0–8.2)	4.1–10.9
Red blood cells (RBC) (M/μl)	5.6 ± 0.3 (4.9–6.3)	5.5 ± 0.3 (4.7–6.4)	4.2–6.3
Platelets (K/μl)	263 ± 60 (153–467)	222 ± 37 (127–296)	140–440
Lymphocytes (%)	37.7 ± 7.8 (23.8–59.3)	28.0 ± 5.7 (18.2–45.6)	10–58.5
Monocytes (%)	6.0 ± 1.0 (3.7–8.3)	5.1 ± 1.3 (3.1–8.6)	0.1–24.0
Granulocytes (%)	56.3 ± 7.8 (36–70.1)	66.9 ± 6.4 (46.8–78.7)	37.0–92.0
Serum aspartate aminotransferase (AST) (U/l)	21 ± 11 (8–70)	21 ± 14 (7–77)	1–37
Serum alanine aminotransferase (ALT) (U/l)	23 ± 24 (3–157)	23 ± 18 (2 – 77)	1–42
Blood glucose (mg/d*l*)	97 ± 14 (72–135)	85 ± 9 (67–101)	70–110
Blood creatinine (mg/d*l*)	1.0 ± 0.1 (0.7–1.3)	1.1 ± 0.1 (0.8–1.4)	0.5–1.5
Blood uric acid (mg/d*l*)	4.9 ± 1.4 (2.8–9.4)	5.8 ± 1.2 (3.7–8.3)	3.5–7.2
Total cholesterol (mg/d*l*)	202 ± 40 (130–282)	*161 ± 36 (87–230)	130–200
Triglycerides (mg/d*l*)	128 ± 73 (44–351)	116 ± 53 (41–230)	36–150

Results are given as means ± SD (range values). *p<0.0001.

Table 2. Semen characteristics of the study population

Characteristics	Exposed group (N=64)	Control group (N=35)	*p
Semen characteristics			
Volume of the ejaculate (m*l*)	3.7 ± 1.8 (1.0–7.0)	3.6 ± 1.5 (2.0–7.6)	0.79
pH	8.1 ± 0.6 (7.0–9.0)	7.7 ± 0.4 (7.0–8.0)	0.004
Sperm concentration (millions/m*l*)	85 ± 34 (20–164)	98 ± 39 (18–182)	0.09
Total sperm count (millions)	300 ± 187 (33–764)	341 ± 181 (62–866)	0.29
Rapid and progressive sperm motility (%)	48.5 ± 17.5 (0–82)	55.0 ± 14.5 (26–82)	0.05
Progressive sperm motility (%)	40.2 ± 13.5 (0–65)	42.1 ± 10.6 (24–64)	0.37
Morphologically normal sperm (%)	52.8 ± 12.3 (23–81)	51.4 ± 11.4 (22–70)	0.58
Multiple anomaly index (%)	47.4 ± 12.3 (22–77)	48.6 ± 11.4 (30–78)	0.62
Live sperm (%)	68.1 ± 10.2 (31–88)	77.3 ± 7.6 (58–89)	<0.0001

Results are given as means ± SD (range values). *Unpaired *t*-test.

Table 3. Distribution of AChE and BuChE activities in the exposed study population according to category of inhibition

Clinical category of AChE and PChE Inhibition (percentage of normal)*	†AChE		††BuChE	
	N (%)	(U/m*l*)	N (%)	(U/m*l*)
Normal (≥75%)	40 (62.5%)	4.1 ± 0.4	8 (12.5%)	2.2 ± 0.2
Mild inhibition (30–74%)	23 (35.9%)	3.1 ± 0.4	55 (85.9%)	1.5 ± 0.3
Moderate inhibition (10–29%)	1 (1.6%)	1.1	1 (1.6%)	0.7
Severe inhibition (<10%)	0	NA	0	NA

*The cut-off points were according to clinical categories of AChE inhibition in acute organophosphorus poisoning, based on the study of Rajapakse *et al*.[28]. Reference values: †4.71 U/m*l*; ††2.55 U/m*l* Results are given as means ± SD. NA: Not applicable.

Table 4. Levels of AChE activity, BuChE activity, sperm DFI, sperm concentration, morphologically normal sperm and live sperm in the exposed study population according to age groups

	18–28 years	29–39 years	≥40 years	Control	*p*
AChE (U/m*l*)	3.7 ± 0.8	3.6 ± 0.5	3.8 ± 0.7	4.1 ± 0.4	0.04
BuChE (U/m*l*)	1.5 ± 0.4	1.6 ± 0.4	1.7 ± 0.4	2.2 ± 0.2	0.003
DFI (%)	35.6 ± 11.7	32.2 ± 9.9	36.6 ± 7.6	24.6 ± 3.2	<0.0001
Sperm concentration (millions/m*l*)	90 ± 39	89 ± 29	62 ± 39	98 ± 39	0.002
Morphologically normal sperm (%)	50.7 ± 16.7	57.2 ± 10.4	45.8 ± 15.8	51.4 ± 11.4	0.05
Live sperm (%)	50.6 ± 15.6	56.5 ±10.2	43.9 ± 14.5	77.3 ± 7.6	<0.0001

Results are given as means ± SD. *Unpaired *t*-test with Bonferroni correction based on 2 outcome measures and 7 predictors, (α=0.05/14=0.00357).

levels. Most of the workers showed normal Tt, PRL, FT4 and TSH levels; however, 44% had increased LH levels and 21% had FSH values above reference limits. No statistically significant associations were observed between serum hormones and cholinesterase levels or semen quality parameters.

Discussion

In Venezuela, the health protection of agricultural workers has been overlooked for many years despite the health risks associated with occupational exposure to agrochemicals. Of main concern are the most frequently used OP and CB pesticides, such as methamidophos, chlorpyrifos, diazinon, ethyl parathion, dimethoate, mancozeb, zineb, carbendazim, carbofuran, propineb and propamocarb. Most of these pesticides belong to WHO Classes Ia (extremely hazardous) and Ib (highly hazardous), which have been either banned or strictly controlled in developed countries. With regard to the most common pesticide-related symptoms, Venezuelan farm workers reported dizziness, headache, nausea, blurring of vision and skin and throat irritation, which were similar to other reports associated with exposure to OP and CB pesticides[29].

Human studies on pesticide exposure and male reproductive and endocrine systems are limited. In this report, our main findings were the following: 1) There was a significant negative association between abnormal BuChE activity and sperm DNA damage in exposed workers; 2) increased sperm DNA damage was associated with significant decreases in the quality of some semen parameters, such as sperm concentration, morphology and vitality; and 3) the levels of Tt, PRL, FT4 and TSH appeared to be normal; however, there was a tendency for increased LH and FSH levels in the exposed study population.

Cholinesterase activity measurements are well-accepted sensitive biomarkers of OP and CB exposure in humans. It has been reported that the half-life of BuChE in the plasma is shorter than that of AChE, which means that BuChE is a more sensitive indicator of the recent pesticide exposure[7, 8]. Our studies showed that a high percentage of the studied farm workers had more inhibited BuChE activity than AChE activity (Table 3); these results were consistent with those reported in other studies[7, 29]. A potential limitation of the present work was the lack of baseline data because the farmers were engaged in intensive agricultural activity all year round. However, the strength of this study lies in the fact that most of the participants (53.5%) were pesticide handlers having worked in agriculture for more than 5 years,

and the majority (75%) had been spraying pesticides at a frequency of one to three times a week (Table 1). From this study, the effect of OP/CB exposure on cholinesterase activity was found predominantly in the inhibition of BuChE activity (Table 3). Overall, these results indicate that a high percentage of the farm workers had chronic OP/CB pesticide exposure. The major factors that could have influenced the high degree of exposure to these pesticides by farm workers were: a) frequency of fumigation, b) duration of exposure of more than two years, c) not using protective equipment and d) use of hazardous pesticides, as gathered from the interviews done with the workers before the blood tests.

Many epidemiological studies have been carried out to investigate the association between occupational exposure to pesticides and the risk of male reproductive toxicity. The integrity of sperm DNA is central to the transmission of genetic information during reproduction, and chromatin abnormalities or DNA damage can result in paternal fertility problems[22, 25, 26, 31]. OPs are considered potent phosphorylating agents, which are potentially genotoxic to animal sperm by altering the chromatin structure via binding to protamines and DNA, causing DNA to become more susceptible to induced denaturation in situ[31]. Our results were consistent with other studies that have associated occupational exposure to OP pesticides with DNA damage in sperm and alterations in semen quality[14, 15, 30]. In the present study, we found associations between BuChE activity and sperm DNA damage, as well as evidence for a relationship between poorer semen quality and increased frequencies of sperm DNA fragmentation. We cannot rule out the possibility that some of our statistically significant or suggestive results were due to chance, since multiple comparisons were made. Nevertheless, these findings may be of concern due to the extensive use of OP/CB, in particular by young workers, placing them at an early age on the edge of sub/infertility[22].

In relation to the assessment of reproductive and thyroid hormone levels in association with occupational pesticide exposure among the farm workers, we found that the levels of Tt, PRL,TSH and FT4 appeared to be normal; however, a high propor-

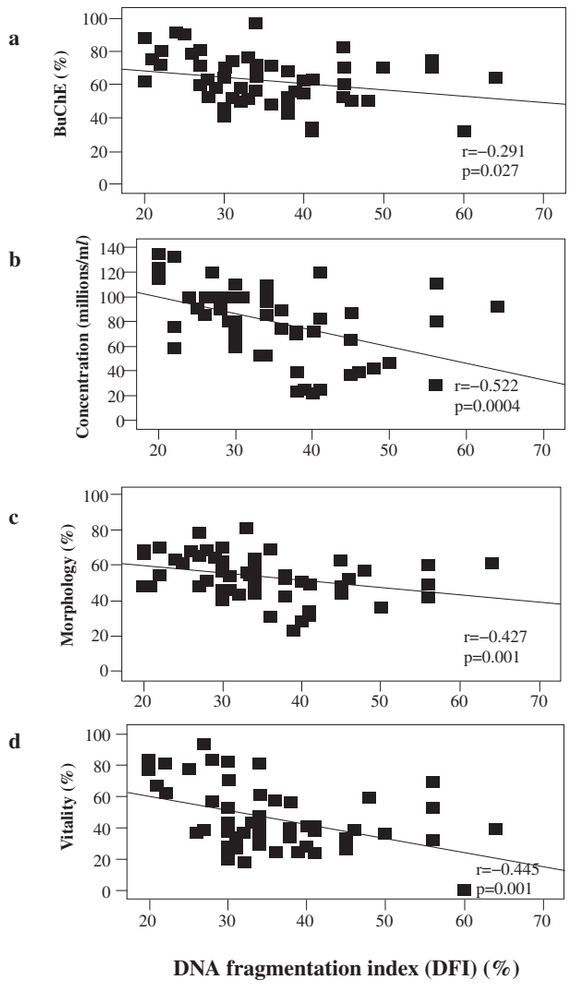

Fig. 1. Correlation between DFI and a) BuChE activity, b) sperm concentration, c) normal sperm morphology and d) sperm vitality in agricultural workers exposed to pesticides.

Table 5. Distribution of reproductive and thyroid hormones in the exposed study population

Hormone	Mean ± SD	Range	Reference values	Percentiles				
				5º	25º	50º	75º	95º
FSH (mIU/m*l*)	7.8 ± 0.5	1.5–24.7	1.5–11.5	2.2	5.0	7.5	10.0	13.4
LH (mIU/m*l*)	10.3 ± 1.1	0.8–43.8	1.2–7.8	1.3	2.4	6.7	15.9	27.9
Prolactin (ng/m*l*)	7.5 ± 3.7	1.9–21.5	3.0–14.7	2.8	4.9	6.8	8.9	15.8
Testosterone (ng/d*l*)	674.7 ± 31.4	320–1,600	262–1,593	380.6	483.0	613.0	806.0	1,216.0
Free T4 (ng/d*l*)	1.3 ± 0.3	0.6–1.9	0.8–1.9	0.9	1.1	1.2	1.5	1.9
TSH (μIU/m*l*)	1.4 ± 0.5	0.8–3.2	0.4–4.0	0.9	1.0	1.2	1.8	2.1

FSH: follicle-stimulating hormone, LH: luteinizing hormone, T4: thyroxine, TSH: thyroid stimulating hormone.

tion (49%) of the farm workers had serum LH levels outside the normal range, with 44% showing increased LH values. On the other hand, 21% of workers had FSH values outside the limits of normality, of which 16% were above the normal limit (Table 5). Our results were in partial agreement with those reported by Padungtod *et al*.[32], who investigated the adverse reproductive effects of OP pesticides among Chinese pesticide factory workers and found a positive association between pesticide exposure and serum LH and FSH levels but a negative association with Tt levels. However, conflicting results were reported in other studies with regard to associations between pesticide exposure and reproductive hormones. In acutely OP-intoxicated individuals, Güven *et al*.[33] reported a significant decrease in serum FSH and an increase in PRL, but no changes were observed in LH levels. Recio *et al*.[34] showed negative associations between OP exposure and serum levels of FSH and LH in Mexican agricultural workers, but they did not observe significant associations between Tt or estradiol serum levels and urinary OP metabolites. In adult men, Meeker *et al*.[35] found an inverse association between urinary levels of OP and CB metabolites and serum LH and Tt levels. In Mexican floriculturists occupationally exposed to OP pesticides, Blanco Muñoz *et al*.[36] demonstrated a negative association between urinary levels of OP metabolites and serum inhibin B, FSH and LH levels, but a positive association with serum Tt levels. Manfo *et al*.[37] did not find significant changes in FSH, LH, T3 and T4 levels in farmers exposed to agropesticides in Djutitsa (West Cameroon); however, there were significant decreases in serum levels of Tt and androstenedione. Overall, these reports indicate that OP and CB pesticides could act as endocrine disruptors in humans. The US EPA defines EDCs as "exogenous agents that interfere with the synthesis, secretion, transport, binding, action or elimination of natural hormones responsible of metabolism, reproduction, development and behavior"[38]. Many chemicals that have been identified as EDCs are pesticides; among them are various OP and CB insecticides, fungicides and herbicides[39]. The toxicity underlying their action could be related to the inhibitory effects of OP pesticides on brain AChE, which may affect the regulation of hypothalamic function by altering the frequency of GnRH pulses that play a critical role in determining the output of LH and FSH from the pituitary[40].

In conclusion, the results of the present study confirm the potential impact of occupational exposure to endocrine disrupting pesticides, such as OPs and CBs, on male reproductive function. Chronic exposure to these pesticides may cause damage to sperm chromatin, decrease semen quality and produce alterations in male reproductive hormones that may lead to adverse reproductive health outcomes. In Venezuela, there is a need to implement a formal worker protection program for agricultural workers, which should include education on the appropriate use of pesticides as well as preventive health monitoring for early detection of pesticide exposure.

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