
Quality spermatic alterations in floriculturists exposed to pesticides in Villa Guerrero, State of Mexico

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Abstract: In Villa Guerrero municipality, floriculture is their first economic activity; there are intensively applied different pesticides alone and in mixtures. They fumigate every day, every second or third day, and men, women, children and third age people participate. The use of prohibited or restricted pesticides such as Tamaron, Temik and Curacron in agricultural activities brings health problems with them, especially in their reproductive health. The aim of this study was to determine whether occupational exposure to pesticides alters semen quality. It was held in an exposed group conformed by 30 male floriculturists and the non-exposed group were 30 university male students. All of the participants gave a semen sample and sign an informed consent. There was made a spermatobioscopy accord to the WHO/PAHO (2010). The spermogram's results in the exposed group showed: significant reduction in the sperm count, in the progressive fast and slow mobility as well as in the normal sperm average. On the other hand, there was a significant increase in the morphologic sperm abnormalities: big, small and double head; short, long and double tale, in comparison with the non-exposed group. Conclusion: it appears that the occupational exposure to pesticides changes the sperm quality.

Keywords: Sperm Quality, Pesticides, Floriculturists

1. Introduction

Nowadays there is a concern about the toxic effects of the utilization of pesticides in agricultural activities that is because of the high number and its diverse chemical composure, as well as to method of use, time of use, inappropriate choice of pesticides, etc [1, 2]. The risk of adverse reproductive and developmental disorders due to occupational pesticide exposure has been evidenced. The male gender is the one in which there has been identified a higher number of reproductive effects by the occupational exposure, being the most important ones: the gonadotropin levels alteration, low seminal volume, reduced sperm count, cytotoxic and cytokinetic alterations, morphologic abnormalities, alterations in mobility and loss of integrity of the sperm membrane, increasing the occurrence of oligozoospermia, teratozoospermia y astenozoospermia, as well as the increase of blood levels of the follicle stimulating

hormone^[1-5]. Meantime, in expose women, the identified effects are variations in hormonal concentrations, spontaneous abortion, congenic defects, delay growth of the fetus and low weight at birth of the product^[6-8].

In flower production, there are used diverse pesticides to counteract plagues, insecticides, fungicides and acaricides mainly. Related to their chemical structure, the most used are benzimidazole, triazole, chlorated, pyrethroids, organophosphates, phenylalanine, dithiocarbamates, imidazole, carbamates and dicarboximide. Between the high and extremely toxic ones there are Tamaron, Temik and Curacron^[9].

The State of Mexico occupies 6,740 hectares (1% of the state territory) in the flower production. The municipality of Villa Guerrero contributes with 80% of the flowers exported to North America and Europe. The floriculture in this entity is the first economic activity, it has the higher number of greenhouses in the state, were there are applied intensively and in mixtures different kinds of pesticides, both in open

environments and in closed ones, it is surrounded by a mountain system, which generates conditions that contributes to the permanence of the substances in the atmosphere. The application of pesticides in mixture form is done every day, each second or third day; it is done by men, women, children and third age people, with the minimum of protection equipment and with total ignorance of the health normativity [9-11].

Finding a relation between the occupational exposure to pesticides with health damage requires to drop out the disciplinary investigation models and begin the bonding between different areas of knowledge that allows a full comprehension of the problem. That is why this study pretends to give information about the possible association between the sperm quality and the occupational exposure to pesticides.

2. Materials and Methods

2.1. Subjects

The exposed group was formed by 30 male floriculturists, from the Villa Guerrero municipality, in the State of México. The non-exposed group was integrated by 30 male members of the Autonomous University of the State of Mexico (UAEMex), Toluca, Mexico. The participation was by invitation and voluntarily, those who accepted signed an informed consent. All participants gave personal and occupational data as well as a semen sample which was held at 37°C until it was processed.

2.2. Spermatobioscopy

It was made according to the established criteria by WHO/PAHO 2010 [12]. The physical and chemical characteristics of the sample were examined: appearance, color, volume, viscosity and liquefaction time.

2.3. Microscopic Examination

Mobility: It was made a fresh preparation of approximately 10 µL of semen in a slide. It was analyzed under the microscope characterizing the movement of 100 spermatozoa and the percentage was reported, taking into account the next criteria: Quick progressivity, a-b grade: Straight, fast movements, travel more than 25µm/s. Slow progressivity, c-d grade: Without sperm progression, but with flagellum mobility or without mobility.

Morphology: A smear was made with 10 µL of semen, there were counted 100 spermatozoa and they're morphologic characteristics were reported by percentage.

- Head: double, big, small, pin.
- Neck: double.
- Tale: double, short, long, without tale.

Spermatozoa count: There was made a dilution 1:20 with Macomber and Saunders solution, a drop was placed in the Neubauer chamber, letting the filling by capillarity to occur, after a few minutes in which the sperm settled the microscopic examination was made. The calculation was made and the spermatozoa number was reported $\times 10^6/\text{mL}$ [12].

2.4. Statistic Analysis

In order to determine the differences of mobility, count, pH and semen volume between the exposed and the non-exposed group, a Mann-Whitney test was made. A Shapiro-Wilk test was made for the differences in the normal and abnormal sperm count between the groups. The Sigma-Plot 12.0 Systat Software Inc. software was used.

3. Results

3.1. Sociodemographic Characteristics

The exposed group had an average age of 29 years old, in a range between 17 and 56 years old, on the other hand, the non-exposed group had an average age of 27 years old, in a range between 18 and 63 years old.

Table 1. Floriculturists' most used pesticides.

Tradename	Active ingredient	Chemical group	Biological activity	Toxicological classification	Frequency use (%)
Lannate	Metomilo	Carbamate	Insecticide	H	83
Manzate 200	Maneb	Dithiocarbamates	Fungicide	S	73
Furadan	Carbofuran	Carbamate	Insecticide	H	73
Tamaron	⊙ Metamidofos	Organophosphate	Insecticide	M	70
Agrimec	Abamectina	Lactone	Insecticide Acaricide	M	67
Ridomil Gold	Metalaxil-M	Acid ester Metilpropionico	Fungicide	S	60
Cascade	Flufenoxuron	Acylureas	Insecticide	S	53
Benlate	Benomilo	Thiocarbamate	Fungicide	S	50
Talstar	Bifrentin	Carboximides	Insecticide	S	47
Nuvacron	⊙ Monocrotofos	Organophosphate	Insecticide Acaricide	H	43
Tecto	Tiabendazol	Benzimidazole	Fungicide	S	43
Pentaclor 600F	⊙ Quintozeno	Chlorobenzene	Fungicide	S	40
Fungicel	Mancozeb	Dithiocarbamates	Fungicide	S	37

⊙Pesticides which are prohibited in other countries but authorized in Mexico.

S= slightly toxic

M=moderately toxic

H= highly toxic

The activities of the exposed group were: planting (17%), pull apart the flower button from the rest (14%), cutting (17%), putting together the packs (15%), spraying (18%), weeding (13%) and others such as watering, fertilize and packing (6%). The most used pesticides for the exposed group were Lannate, Manzanate 200, Furadan, Tamaron, Agrimec and Ridomil gold. The application was in mixture the most of the times, those were form by different biological activity compounds, different chemical group and different toxicity, the Table 1 shows the most used pesticides. Talking about the protection equipment used for the flower growing, 41% if the individual wore boots, 23% wore apron, 18%

wore mask and 9% wore gloves, whereas the non-exposed group activities were mostly academic activities.

3.2. Spermograms

In the spermograms there were found significant differences between the exposed and the non-exposed group in the progressive mobility (PRM), slow progressive mobility (SPM), sperm average count and normal and abnormal percentage. While in the pH and seminal volume, there were no significant differences between the groups. The data details are represented in Table 2.

Table 2. Results and statistics analysis of the physical and chemical characteristics of the semen samples of the groups.

Group	Mobility		Count	pH	Volume	Spermatozoa	
	PRM %	SPM %	No. sperm 10 ⁶ /mL		mL	Normal %	Abnormal %
Exposed	71*	29*	61*	8	2.0	46.5*	53.5*
Non-exposed	86	14	84	8	2.3	59.0	41.0

* Significant difference, exposed versus non-exposed, Mann-Whitney's U test, $p < 0.001$

On the other side, the evaluation of morphological abnormalities in the sperm was made, finding significant differences in parameters as head size increased, head size decreased, double head, double tale, short tale and long tale,

between the exposed group and the non-exposed group. At the same time, for the pin head, double neck and without tale, there were no significant differences. All of the results are represented in Table 3.

Table 3. Results and statistical analysis of the abnormalities in the spermatozoa.

Group	Head				Neck	Tale		
	Macro	Micro	Pin	Double	Double	Short	Long	Withno
Exposed	9.5*	8*	9.0	2.0*	1.93	8.0*	10.0*	1.0
Non-exposed	7.0	5	10.0	1.0	1.90	4.0	7.0	1.0

* Significant difference, exposed versus non-exposed, Shapiro-Wilk, $p < 0.001$

4. Discussion

The result show that more than 40% of the workers of the study use pesticides such as Furadan, Tamaron, Nuvacon and Pentaclor 600F, which have been restricted in their application by the European Union and the Environmental Protection Agency (EPA) [13]. This is due to their high toxicity levels and their impact in the human health, since overexposure to Furadan, Tamaron and Nuvacon triggers headache, dizziness, respiratory difficulty, abdominal spotting, nausea, salivation, blurred vision, dilated pupils, runny nose and mouth, skin bruising, convulsions, tremor, coma and death, as a result of inhibition of acetylcholinesterase or erythrocyte acetylcholinesterase. On the other hand, the Pentaclor 600F, organochlorided pesticide, highly persistent in the environment, it is not clear risk to human health [14-16]. Although existing information on the damage to health caused by these pesticides regulations Mexico has not restricted its use [17, 18].

These floriculturists are also exposed to Lannate, Manzate, Benlate and Tamaron of which it is known that: methomyl (Lannate®) is a carbamate, and is known to be environmental estrogen which alters the reproductive system, increasing the abnormal sperm count in mice [14, 19]. Mancozeb (Manzate®) is a dithiocarbamic fungicide, increases the number of

abnormal sperm in mice, and benomyl (Benlate®), a benzimidazolic fungicide, is known to act as an environmental estrogen decreasing testes' weight, sperm count and causes degeneration of the testes in mice [14, 20].

The results show the detrimental effects of pesticides on the germ cells, affecting male sperm parameters, so that the sperm may have less probability of a fertilization, fact consistent with other studies such as Hauser *et al.*, (2003), who found a direct relationship between increased environmental exposure to pesticides and less mobility, concentration and percentage of spermatozoa [21], and Abell, *et al.*, (2000), who discussed the role of chemical exposure in the workplace on male reproductive function, associating it with semen quality, finding that spermatogenesis is severely affected by exposure to pesticides in handling greenhouse crops [22].

In this study, there was significant difference in the parameters of sperm quality, finding decreased sperm count, mobility, as well as the number of normal and abnormal sperm and increased structural abnormalities of spermatozoa among the exposed group versus the no exposed. In spermatogenesis, whether pesticides damage to the immature cell, this can cause the damaged sperm cell is removed, so that only those with no apparent damage may mature and eventually leave with the ejaculation, which explains the

decrease in sperm count^[23, 24].

Because of this, it must be remembered that the ultimate demonstration of an association between pesticides acting as endocrine disruptors and in generation of reproductive toxicity effects is not immediate, given the difficulty of developing multidisciplinary epidemiological studies, where the cause-effect may be altered over time. Therefore, studies such as this, should be favored, and above all, the continuity of the same, trying to identify the cause-effect relation by the use of pesticides with greater certainty. Meanwhile, it is proposed, to pay attention to the reasonable doubt instructions and the precautionary principle to act preemptively without waiting for irrefutable evidence. Otherwise, the consequences for animal and human health could be dramatic and irreversible.

5. Conclusion

The scientific evidence show the negative impact of the pesticides in the occupationally exposed population, as these affects acutely and chronically the male reproductive organs. This research contributes, giving another evidence of alterations in sperm quality induced by exposure to pesticides, in this case, due to occupational exposure with scant protective equipment, with use of prohibited or restricted products internationally and also employing mixtures thereof.

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