IMPACTS OF PESTICIDE USE ON SEMEN CHARACTERISTICS AMONG RICE FAMERS IN KIENXUONG DISTRICT, THAIBINH PROVINCE, VIETNAM

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Abstract. This case-control study assessed the effects of pesticide use on semen characteristics among rice farmers of Kienxuong District, Thaibinh Province, Vietnam. Semen samples of 1,036 rice farmers were obtained by manual masturbation and screened at Commune Health Stations. Of these, 156 abnormal semen samples were identified; 314 rice farmers with normal semen were recruited as controls. The semen characteristics (volume, sperm concentration, total sperm count, motility, vitality and morphology) of the cases were considerably poorer than the controls. Factors associated with abnormal semen after adjusting for age, smoking and alcohol drinking by logistic regression were: distance of less than 300 meters from household to rice fields and duration of work over 10 years as a farmer (adjusted OR = 3.16, 95% CI: 1.97-5.05 and adjusted OR = 3.98, 95% CI: 2.20-7.21, respectively). Rice farmers without personal protective equipment (PPE) when spraying pesticides and without pesticide training (adjusted OR = 3.05, CI: 1.92-4.85 and adjusted OR = 1.90, CI: 1.14-3.16, respectively) were also at risk for abnormal semen compared to controls. These findings showed the strength of association between pesticide use and abnormal semen characteristics among rice farmers in Kienxuong District, Thaibinh Province, Vietnam.

INTRODUCTION

Reduction of sperm count, lower motility and lower normal sperm morphology are associated with a higher probability of infertility (Anne, 2000; ADAM, 2003). The adverse effects of pesticides on the male reproductive system, especially semen characteristics, is a major health problem all over the world. World pesticide use is high for all pesticide types (EPA, 2001). Imported pesticides have increased significantly in Southeast Asia (FAO, 2005; Hoe, 2005) and Vietnam (Trung and Ky, 2001; Hoe, 2005). Results from several scientific studies have indicated temporal and regional changes in male reproductive health. Additionally, a possible decline in semen characteristics has been observed and reported in many studies (Leto and Frensilli, 1981; Auger *et al*, 1995; Swan *et al*, 1997). Experts in male fertility and epidemiologists (Auger *et al*, 1995; Fisch and Goluboff, 1996; Irvine *et al*, 1996; Swan *et al*, 1997, 2000; Emanuel *et al*, 1998) have extensively explored and disputed the findings of Carlsen *et al* (1992), who showed a significant decline in semen quality over the past 50 years on meta-analysis of 61 studies.

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Several international studies have shown evidence of reduction in semen quality or lower sperm counts due to agricultural pesticides. (Wyrobek *et al*, 1981; Bigelow *et al*, 1998; Padungtod *et al*, 1999, 2000; Tan *et al*, 2002; Marmol-Maneiro *et al*, 2003; Swan *et al*, 2003; Kamijima *et al*, 2004; Meeker *et al*, 2004; Perry *et al*, 2006). Based on these findings, we conducted a case-control study to identify the impact of pesticide use on semen characteristics among rice farmers in Kienxuong District, Thaibinh Province, Vietnam.

MATERIALS AND METHODS

Approval of this study was obtained from the Thaibinh Medical University Ethics Committee, Vietnam. Before being enrolled in the study, male farmers were informed of the purpose of the study, procedures, benefits and possible risks involved. Male farmers were also told that all information and biological samples obtained in this study would remain confidential, and they had the right to refuse to participate and could terminate the participation at any time. All of the participants in the study were required to sign a consent form prior to their participation.

This study was conducted among male farmers from 5 communes in Kienxuong District, Thaibinh Province, Vietnam. The total number of rice farmers asked to participate in the study was 1,655, of whom 1,036 (62.59%) consented and successfully provided semen samples.

Semen screening

The inclusion criteria for enrollment of subjects were rice farmers 25-40 years old, living in Kienxuong District, Thaibinh Province for more than 5 years, not currently suffering from any acute infection, or chronic diseases (*eg* tuberculosis, sexually transmitted diseases, cancer, mental diseases), and having no problems with erection or a history of vasectomy.

Case and control definition

Semen analysis was performed according to the WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction (WHO, 1992) to classify the cases (abnormal semen) and controls (normal semen). Abnormal semen was confirmed by one of the following criteria: sperm concentration less than 20 x 10^6/ml, fewer than 50% of spermatozoa with rapid and slow progression, fewer than 25% of spermatozoa with rapid progression, fewer than 30% of spermatozoa with normal morphology or the absence of spermatozoa in the semen. Controls were those subjects without the above sperm characteristics.

Semen collection and analysis

A semen sample was collected by masturbation into a sterile wide-mouth glass container in a mobile laboratory at each Commune Health Station. Sexual abstinence of 2-3 days was adequate for the accurate assessment of semen quality. Information on date, time, spillage and number of days since last ejaculation were recorded and coded on each sample. The samples were kept in an incubator at 37ºC until liquefaction and subsequently examined. All semen samples were processed and analyzed by one group of experienced laboratory medical doctors of Thaibinh Medical University, Vietnam. Volume, sperm concentration, total sperm count, proportions of motility, sperm vitality and proportions of morphology were examined (WHO, 1992).

The semen samples were liquefied completely and mixed thoroughly. A drop was applied onto a clean glass slide and mounted with a glass cover and examined at 400 x magnification. An improved Neubauer counting chamber (hemocytometer) was used to determine the sperm concentration.

Sperm motility was graded as 0-4 according to the quality of forward progression in the majority of the sperm: rapid progres-

sion, slow progression, non-progression and immotile. In the measurement of motility, at least 200 sperm were counted. The vital stain method was used to identify sperm vitality (Eosin Y test), the Giemsa stain method was used to observe the sperm morphology in order to obtain the proportion of normal sperm, head defects, neck and mid-piece defects and tail defects (WHO, 1992).

Interviewing the subjects

A questionnaire was designed to record a history of pesticide exposure and other potential risk factors among farmers in the previous year. Exposure was defined as exposure to pesticides in the agricultural setting.

Population profile (age, marital status, educational level, family members, family income, and residency), exposure pattern (type of pesticide use, duration of use, duration of work, PPE use when mixing, spraying and cleaning pesticide cans and other risk behaviors (smoking, tea drinking and alcohol drinking) were obtained.

Statistical analysis

Log transformation was applied to improve the data normality of some of the semen characteristics when necessary. Descriptive data and *t*-test analyses were performed. Univariate and multivariate analyses were used to assess the potential risk factors for abnormal semen. The crude odds ratio (OR) with its 95% confidence interval (95% CI) and adjusted OR with its 95% confidence interval (95% CI) (adjusting for age, smoking, alcohol drinking and tea drinking) were calculated for case-control associations with factors suspected of resulting in abnormal semen. Logistic regression was performed by STATA 9.0 software.

RESULTS

Table 1 shows the demographic characteristics of 156 cases and 314 controls. The

Table 1
Population profile of male farmers.

Variable	Cases (n=156)		Controls (n=314)	
	n	%	n	%
Age group (years)				
25-30	42	26.92	92	29.30
31-35	37	23.72	77	24.52
36-40	77	49.36	145	46.18
Mean age (years)	34.43 ± 4.88		34.04 ± 4.99	
Educational level				
Primary school	8	5.13	17	5.41
Secondary school	116	74.36	243	77.39
High school	32	20.51	54	17.20
Distance from household to the rice field				
Over 600 meters	10	6.41	49	15.61
301-600 meters	34	21.79	123	39.17
Under 300 meters	112	71.79	142	45.22
Smoking				
No	62	39.74	181	57.64
Yes	94	60.26	133	42.36
Alcohol drinking				
No	51	32.69	158	50.32
Yes	105	67.31	156	49.68

Table 2
Semen characteristics of male farmers.

Semen characteristics	Cases (n=156)		Controls (n=314)		WHO standard parameters	p-value
	Geometric mean	Range	Geometric mean	Range		
Volume (ml)	1.06	0.5 -3.8	1.76	1.2 -7	≥ 2	0.000
Concentration (10^6/ ml)	11.21	4 -19.8	54.74	20 -139.6	≥ 20	0.000
Total sperm count (10^6)	11.92	2 -49.4	96.41	24 -756.3	≥ 40	0.000
Motility (%)						
Rapid progression	14.46	0 -45	42.54	25 -85	≥ 25	0.000
Slow progression	15.46	0 -35	17.66	5 -40	-	0.018
Rapid and slow progression	30.94	0 -75	61.15	50 -90	≥ 50	0.000
Non-progression	14.05	0 -42	13.07	2 -35	-	0.000
Immotile	44.79	0 -98	21.11	1 -40	-	0.000
Vitality (%)	44.34	0 -82	65.84	40 -90	≥ 75	0.000
Morphology (%)						
Normal morphology	70.64	40 -95	77.62	37 -95	≥ 30	0.000
Head defects	13.81	0 -43	10.83	1 -33	-	0.000
Neck and mid-piece defects	4.21	0 -16	3.72	0 -21	-	0.430
Tail defects	6.93	0 -43	4.31	0 -23	-	0.000

mean age was similar between cases and controls (34.43 ± 4.88 years and 34.04 ± 4.99 years, respectively). The percentage of rice farmers from 36 to 40 years old was higher than others in both cases (49.36%) and controls (46.18%). The majority of the cases and controls had an educational level of secondary school (74.36% and 77.39%, respectively). Distance less than 300 meters from the household of farmers to the rice fields was more common among cases than controls (71.79% and 45.22%, respectively). Smoking and alcohol drinking among cases were higher than controls (60.26% versus 42.36% and 67.31% versus 49.68%, respectively).

All the semen characteristics of the cases were significantly poorer than controls ($p< 0.01$). In the abnormal group, volume (1.06 ml), concentration (11.21 x 10^6/ ml), total sperm count (11.92 x 10^6 sperms), motility, rapid progression (14.46%), rapid and slow progression (30.94%) and vitality (44.34%) (except percentage of normal morphology) were all lower than the normal parameters of the World Health Organization criteria (1992). All geometric means of semen characteristics in the controls (except volume) were above World Health Organization criteria (Table 2).

The factors associated with abnormal semen in rice farmers are presented in Table 3. Factors for abnormal semen were: distance less than 300 meters from household to rice fields (crude OR = 3.08, 95% CI: 2.03-4.66), duration of work as a rice farmer over 10 years (crude OR = 3.61, 95% CI: 2.18-5.99), no pesticide training (crude OR = 2.49, 95% CI: 1.58-3.92), rice farmers without PPE when mixing pesticides (crude OR = 1.55, 95% CI: 1.05-2.30), and without PPE when spraying pesticides (crude OR = 3.02, 95% CI: 2.01-4.54). The factors were significantly associated with abnormal semen after adjusting for age, smoking, and alcohol drinking by logistic regression. Distance less than 300 meters from household to rice field and duration of work over 10 years as a farmer were highest

Table 3
Factors associated with abnormal semen in male farmers.

Factors	Cases (n=156)	Controls (n= 314)	Case rate (%)	Crude OR	95% CI	Adjusted OR[a]	95% CI
Distance from household to rice fields							
>300 meters	44	172	20.37				
≤300 meters	112	142	44.09	3.08	2.03-4.66	3.16	1.97-5.05
Duration of work							
<10 years	22	117	15.82				
≥10 years	134	197	40.48	3.61	2.18-5.99	3.98	2.20-7.21
Pesticide training							
Yes	31	120	20.52				
No	125	194	39.18	2.49	1.58-3.92	1.9	1.14-3.16
PPE when mixing pesticides							
Yes	87	208	29.49				
No	69	106	39.42	1.55	1.05-2.30	0.98	0.62-1.56
PPE when spraying pesticides							
Yes	48	180	21.05				
No	108	134	44.62	3.02	2.01-4.54	3.05	1.92-4.85

[a] Adjusted for age, smoking and alcohol by logistic regression.
PPE – personal protective equipment.

risk for abnormal semen (adjusted OR = 3.16, 95% CI: 1.97-5.05 and adjusted OR = 3.98, 95% CI: 2.20-7.21, respectively). Rice farmers without PPE when spraying pesticides and no pesticide training (adjusted OR = 3.05, 95% CI: 1.92-4.85 and adjusted OR = 1.90, 95% CI: 1.14-3.16, respectively) were also at risks for abnormal semen compared to controls.

DISCUSSION

To our knowledge, this is the first study of semen characteristics among rice farmers at the community level in Kienxuong District, Thaibinh Province, Vietnam. It included a large number of cases (156) and controls (314). The findings are likely to accurately reflect semen characteristics of rice farmers because of the large number of participants. The major findings of our study were that pesticides use was significantly associated with abnormal semen characteristics. Distance less than 300 meters from household to rice fields, duration of work as a farmer for over 10 years and farmers without PPE when spraying pesticides were strong risk factors for having abnormal semen.

On semen analysis, the WHO laboratory manual for the examination of human semen was followed strictly (WHO, 1992). Only one highly trained technician and one health assistant were employed. Semen were evaluated within 60 minutes of collection. Each semen sample was kept in a special portable incubator at 37ºC for liquefaction, which took about 20 minutes. Therefore, misclassification was kept to a minimum.

In the interview phase, related biases were ruled out because the interviews were performed blindly and interviewers did not know the semen characteristics of the rice farmers before being interviewed. Concerns about recall bias are more likely to be overrated in case-control studies and misclassification is a major limitation in many investigations of pesticides use in order to extrapolate exposure. However, these arguments were reduced because the interviewers tried to obtain information that

was as specific as possible from male farmers by the detailed questionnaire.

The usefulness of our study may be affected by the variability of semen characteristics. This probably reduces the ability to discriminate between farmers with normal and abnormal semen characteristics because only one semen sample was collected per subject. After adjusting for potential confounders, the biological variability of semen characteristics remained uncontrolled. Many human semen studies have shown that semen characteristics fluctuate daily, weekly and yearly (Auger *et al*, 1995; Ombelet *et al*, 1996).

The finding that male farmers exhibited abnormal semen characteristics after pesticide use should contribute significantly to pesticides regulations, especially to the requirement of PPE for farmers. Further studies with multiple semen samples per subject will characterize more accurately the semen characteristics to obtain conclusive findings.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education and Training, Vietnam. The authors would like to thank all the health workers of the Commune Health Stations in Kienxuong District, Thaibinh Province and our colleagues in Thaibinh Medical University, Vietnam who helped to complete the data collected for this study. We are grateful to the male farmers who participated.

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