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Depression of Sperm Counts Among Agricultural Workers Exposed to Dibromochloropropane and Ethylene Dibromide

Wataru Takahashi*, Lyle Wong, Beverly J. Rogers, and Ralph W. Hale

Pesticide Hazard Assessment Project, Pacific Biomedical Research Center, University of Hawaii and Department of Obstetrics & Gynecology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822

A high degree of abnormal sperm morphology was found among 4 allegedly infertile residents of Molokai reporting to the Ob-Gyn Clinic with no occupational exposure to toxic agents known to affect spermatogenesis. The reason for the high rate of abnormality is this group was not known, but evidence of extensive seminal pathology in these men suggested that some unknown environmental factor may be adversely affecting spermatogenesis. This pilot study was undertaken to investigate spermatogenesis among men in this community.

Background

Molokai, the fifth largest island of the Hawaiian chain, has a land area of 679 km^2. The estimated population of Molokai is about 5,300 people, representing 0.6 % of the total state population.

The livebirth rate for the Molokai population in 1977 was 17.9 per 1,000 civilian population, compared with 18.9 for the state population. The death rate of infants under 1 year of age for Molokai in 1977 was 10.1 per 1000 livebirths and 11.6 per 1000 livebirths for the state. Fetal death rate was 48.1 per 1000 deliveries, compared with 85.5 for the state population. Trends in livebirths for the Molokai population during the period 1957 to 1977 are comparable to the rates for the entire state during this same period. Since 1968 the death rates for infants under 1 year of age for Molokai have averaged below the state level. Examination of recent trends in fetal death rate also reveals that the rate for Molokai has been lower than that of the entire state since 1959.

Several public and private water systems consisting of high level mountain sources upgradient of the pineapple fields service the communities of Molokai.

Industry

Agriculture is the principal industry on Molokai. The major crop is pineapple with 1,400 ha under cultivation by the Del Monte Corporation. Diversified agriculture accounts for an additional 140 ha with about 120 ha in vegetable crops. Cattle is raised on approximately 50,000 ha.

Mailing Address: * Wataru Takahashi, Pesticide Hazard Assessment Project, Pacific Biomedical Research Center, University of Hawaii, 1997 East West Road, Honolulu, HI 96822.

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Pesticide Usage

The pineapple industry is the largest user of pesticides on Molokai. The following pesticides are used: DBCP, EDB, benomyl, captafol, captan, ethephon, ethylene, ametryn, atrazine, bromacil, dalapon, diuron, diazinon, heptachlor, malathion, oxamyl, and phenamiphos. Pesticides used in diversified agriculture (mainly seed corn) include the following: aldicarb, diazinon, methomyl, methyl parathion, metasystox-R, monocrotophos and Dithane M-45. The cattle industry uses coumaphos, malathion, crufomate, dioxathion, dichlorvos, and 2,4-D.

DBCP and EDB are the only chemicals in the foregoing list that are presently known to affect spermatogenesis. The Del Monte Corporation used approximately 19,000 kg of DBCP and 24,000 kg of EDB on Molokai in 1979.

METHODS

Selection of subject

Volunteers were solicited from three groups of non-vasectomized men consisting of agricultural workers, allegedly infertile men seen at the Molokai Obstetrics and Gynecology Clinic, and men from the general population of Molokai.

Examination procedure

Each volunteer was asked to complete a medical history questionnaire, and information on the participant's reproductive system was obtained with particular attention given to factors reported to affect spermatogenesis, i.e., recent febrile illness, venereal disease, excessive alcoholic intake, and specific drugs. Occupational and pesticide exposure histories were also obtained from each participant by interview.

Semen collection and analysis

Sperm samples were obtained by masturbation at home or at the hospital after a minimum of 48 h of sexual abstinence and taken to the laboratory within an hour. A single specimen was collected from each of 32 men and the sperm count, morphology and motility determinations were usually done 0.5 h (but no later than 1.0 h) after collection. After completion of sperm assessment, each sample was washed and resuspended in BIGGERS, WHITTEN & WHITTINGHAM'S medium (BIGGERS et al. 1971) for shipment to the laboratory of the Kapiolani-Children's Medical Center for differential morphologic assessment. Smears were then made and stained by the Papanicolaou technique for differential analyses.

Estimation of exposure

The pineapple fields were fumigated with DBCP and EDB from May through December 1979. Drip irrigators applied DBCP and EDB for a

total of 6 days during the period 22 May to 5 July 1979. The field research workers used DBCP and EDB 5 days during the period 16-23 August 1979. Semen samples were collected from October 1979 through January 1980. Because precise data on the levels of DBCP in the working environment of the pineapple workers were not available, estimation of individual worker's exposure to this chemical was not possible. Moreover, the reported use of protective clothing and equipment by these men during the handling of DBCP made quantification of exposure impossible.

Statistical Analyses

Evaluation of the data collected was carried out by non-parametric techniques (HOLLANDER & WOLFE 1973) using chi-square tests and Dunn's multiple comparison test. Statistical comparisons were also performed between the study population (agricultural and reference groups) and the "fertile" and "infertile" Honolulu populations of ROGERS et al. (1979).

Water sampling sites

Samples were collected from three sources of domestic water supply: (a) Kaunakakai; (b) Hawaiian Homes Commission at Hoolehua; and (c) Kualapuu. A sample was also collected from a shallow open well along the South Central coast about 0.6 km along the ground flow path downgradient of pineapple fields. All samples were analyzed by the project laboratory. The presence of DBCP and EDB was determined by codistillation of water samples with ethyl acetate by a method giving a minimum detection limit of 1.0 ppb.

RESULTS

Study population

Semen analyses were performed on 13 pineapple field workers, and a reference group of 19 men (including one infertile male) from the general population. The pineapple field workers consisted of a formulator, drip irrigators, agricultural research workers and other workers potentially exposed to DBCP, including a planter, spray boom operator, equipment serviceman and truck driver. The ages of these men ranged from 23 to 51 years, with a mean of 37.6. All had previously been exposed to DBCP. Their exposure to DBCP averaged 2.8 years with a median frequency of 6 days per year. Ten of the pineapple workers were married, one was a widower, and 2 were single. Seven of the married men and the widower had fathered children. There were 5 miscarriages in 22 pregnancies among the spouses of these workers.

The reference group was composed of the unemployed, clerical, skilled and unskilled, technical and kindred workers. Their ages varied from 21 to 49 years, with a mean age of 32.4. Four men had previously worked in the pineapple industry (the most recent in 1976), but none of these men had been exposed to DBCP or EDB. Ten men in the reference group smoked marihuana. The frequency of

smoking ranged from occasional to 15 "joints" of marihuana per day, including one azoospermic who smoked 2 "joints" of marihuana per day for 14 years. In contrast, only one pineapple worker smoked one "joint" of marihuana per week.

There were 13 married men in the reference group. Nine of the married men and one single person had fathered children. There were 5 miscarriages in 21 pregnancies among the spouses of the married men. Three miscarriages in 3 pregnancies involved one couple. Data from 14 individuals in the reference group are evaluated in this report: excluded from comparative analyses were 5 persons who smoked 2 or more "joints" of marihuana per week.

Sperm Counts

The sperm counts of the agricultural group ranged from 6.5 to 84 million/mL, with a mean of 33.5 million/mL and a median of 31.5 million/mL. The sperm concentration of the reference group (minus 1 azoospermic and 4 other marihuana smokers) varied from 7 to 240 million/mL, with a mean of 84.9 million/mL and a median of 69.2 million/mL. Three of 13 individuals (23%) of the agricultural group, and 2 of 14 men (14%) of the reference group had counts below 20 million/mL. None of the agricultural group had counts exceeding 100 million/mL, while 5 of 14 men (36%) of the reference group had counts greater than 100 million/mL. The comparative distribution of these observations is shown in Table 1.

Examination of the frequency distribution of sperm count indicated significant differences among the agricultural, reference and fertile Honolulu populations ($P<0.001$). Significant differences were also observed between the agricultural and reference groups ($P<0.02$), and between the agricultural and fertile Honolulu population. There was no significant difference in the sperm count distribution between the reference group and the fertile Honolulu population.

The sperm counts of those who smoked 2 or more "joints" of marihuana per week ranged from 1 to 67 million/mL (excluding one azoospermic), with a group mean of 17.8 million/mL and a median of 1.5 million/mL, whereas the mean for the occasional user was 81 million/mL with a median of 89 million/mL, and that of the non-user was 87 million/mL with a median value of 59 million/mL. The results of semen analyses of those who smoked 2 or more "joints" of marihuana per week are presented in Table 2.

Sperm volume and morphology

The mean ejaculate volume of the agricultural group was 2.67 mL, whereas that of the reference group was 2.80 mL. The percentage of sperms with oval (normal) heads in specimens from the agricultural group ranged from 46.5 to 81% with a median of 60.0%, compared with a range of 19.5 to 75% and a median of 54% for specimens from the reference population. The comparative

Table 1. Comparative distribution of sperm counts

	Pineapple workers	Reference group[a]	Population: Fertile[b]	Population: Infertile[c]
Number of men	13	14	72	72
Mean age	37.6	32.4	-	-
Mean sperm volume, mL	2.67	2.80	-	-
Mean count million/mL	33.5	84.9	106.4	48.1
Median count million/mL	31.5	69.2	82.8	29.0
% counts (million/mL)				
<10	15.4	7.1	0	13.9
10.1-20	7.7	7.1	5.5	23.6
20.1-40	53.8	0	12.5	27.8
40.1-60	15.4	28.6	11.8	13.9
60.1-100	7.7	21.4	27.8	12.5
>100	0	35.7	43.1	8.3

[a] Minus azoospermic and marihuana users (2 or more "joints" per week).
[b] Fertile Honolulu population (ROGERS et al. 1979).
[c] Infertile Honolulu population (ROGERS et al. 1979).

Comparison			Results
Agriculture vs. Reference	X^2	= 8.13	P<0.02 Signif.
Agriculture vs. Reference & Fertile	X^2	= 364	P<0.001 Signif.
Agriculture vs. Fertile	z	= 15.43	Signif.
Agriculture vs. Infertile	z	= 0.27	N.S.
Reference vs. Fertile	z	= 0.66	N.S.

Table 2. Sperm concentration, motility and morphology in semen of five marihuana smokers

I.D. No.	Age	Volume in mL	Sperm conc. million/mL	Motility %	Grade of activity	Normal %	Number of "joints"/week
800	35	2.3	0	-	-	-	14
803	23	1.2	<1	0	1	4	2-3
807	20	2.5	67	45	4	69	14
809	22	1.8	1	-	2	28	75
812	21	0.4	2	<10	1	9	2

distribution of sperm morphology is presented in Table 3. Differences in distribution between the agricultural and reference groups were not significant, but the distribution of the reference group differed significantly from that of a fertile Honolulu population as shown in this table.

Table 3. Comparative distribution of normal sperm

Group	Number of men	<39		40-59		60-79		>80	
		No.	%	No.	%	No.	%	No.	%
Agricultural workers	13	0	0	6	46.1	5	38.5	2	15.4
Reference group[a]	14	4	28.6	4	28.6	6	42.8	0	0
Fertile population[b]	72	5	6.9	16	22.2	27	37.5	24	33.3
Infertile population[c]	72	33	45.8	19	26.4	15	20.8	5	6.9

[a] Minus azoospermic and marihuana users (2 or more "joints" per week)
[b] Fertile Honolulu population (ROGERS et al. 1979).
[c] Infertile Honolulu population (ROGERS et al. 1979).

Comparison	Results	
Agricultural + Reference vs. Fertile	$X^2 = 14.54$ $P<0.01$	Signif.
Agricultural vs. Reference	$z = 1.22$	N.S.
Agricultural vs. Fertile	$z = 1.66$	N.S.
Reference vs. Fertile	$z = 3.41$	Signif.

Evaluation of sperm morphology among the marihuana smokers revealed that the mean percentage of normal heads in specimens from those who smoked 2 or more "joints" of marihuana per week was 28% with a median of 18% (excluding the azoospermic), compared with a mean of 55% and a median of 63% for the occasional users. The median frequencies of tapered and immature sperms among specimens collected from the reference and agricultural groups were 20% and 4%, and 13% and 0.8%, respectively. In contrast, the mean frequency of tapered sperms in MACLEOD and GOLD'S "fertile" population was 4% (MACLEOD & GOLD 1951). Immature sperms are seldom seen under normal conditions. Increase in tapered and immature sperms is reported to occur in human seminal cytology under stress (MACLEOD 1962, 1967).

Sperm motility

The percentage of motile sperms among specimens from the agricultural workers ranged from 30 to 90% with a median of 75%; that of the reference group varied from 50 to 95% with a median of 70%. The quality of sperm motility in specimens from the agricultural group ranged from grade 2 to 4, with a mean of 3.38, whereas that of the reference group varied from grade 3 to 4 with a mean of 3.67.

Determination of DBCP and EDB in water samples

The results of analyses of domestic water and well water samples were negative for DBCP and EDB.

DISCUSSION

Examination of the vital statistics did not reveal any apparent problems with fertility, infant mortality or birth defects among the population of this community.

Marked impairment of spermatogenesis observed in samples from 5 men who were frequent users of marihuana suggests the possibility that the high rate of sperm abnormalities initially found among 4 allegedly infertile men, one of whom has since fathered a child, may have been due to the adverse effects of frequent marihuana use. Marihuana smoking in men has been reported to reduce testosterone levels (KOLODNY et al. 1974), and can lead to a depression of sperm counts in both rats and man (HUANG et al. 1979, HEMBREE et al. 1979).

The pineapple workers were exposed to many different pesticides, but DBCP is the only chemical known to affect human spermatogenesis. Although precise DBCP exposure data could not be obtained, depression of sperm counts among these workers appears to be consistent with the results of other studies among farmer workers exposed to DBCP (GLASS et al. 1979, SANDIFER et al. 1979). The gonadotoxic effect of DBCP has been well documented among workers in chemical production plants (LIPSHULTZ et al. 1980, MARSHALL et al. 1978, WHORTON et al. 1979).

Analyses of data in this severely limited sampling revealed significant differences in the distribution of sperm counts between the pineapple field workers and the reference population. Twenty-three percent of the agricultural workers were oligospermic (counts below 20 million/mL) and 54% had low normal counts (20-39 million/mL), whereas 14% of the reference group were oligospermic and none had counts betwen 20 to 39 million/mL.

The findings of this study are suggestive of gonadal toxicity from exposure to DBCP, and indicate that more extensive investigation of spermatogenesis among other pineapple field workers exposed to DBCP should be undertaken.

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