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• *Original Contributions*

SEMEN CHARACTERISTICS OF VIETNAM VETERANS

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Abstract — As part of an epidemiologic study of the health status of a randomly selected group of Vietnam veterans, we measured the semen characteristics of 324 Vietnam veterans and compared them with a similar group of 247 veterans who did not serve in Vietnam. The participation rate was 81% in both groups. Measurements of sperm concentration, movement characteristics, and head dimensions were performed, using the Cellsoft computer-assisted semen analysis system. We found that Vietnam veterans had significantly ($p < 0.05$) lowered mean sperm concentrations (64.8×10^6 sperm/mL for Vietnam veterans vs 79.8×10^6 sperm/mL for non-Vietnam veterans), and Vietnam veterans were twice as likely to have sperm concentrations ≤ 20 million/mL (odds ratio = 2.7, 95% confidence interval = 1.3–5.7). Vietnam veterans also had a significantly lowered mean proportion of morphologically normal sperm heads (57.9% vs 60.8%), with significantly longer mean major axis length and head circumference. The proportion of motile cells, velocity, linearity, amplitude of lateral head displacement, and beat frequency were not different between the two groups. Despite differences in sperm characteristics, Vietnam and non-Vietnam veterans reported fathering similar numbers of children.

Key Words: Vietnam veterans, Semen, Sperm, Fertility, Epidemiology, Sperm concentration, Sperm morphometry, Sperm motility.

INTRODUCTION

Vietnam veterans have expressed concern about adverse reproductive effects, including impotence, loss of libido, infertility, birth defects, and neonatal death, resulting from military service in Vietnam and possible exposure to Agent Orange (1). Of these potential effects, the two that have received the greatest attention are adverse pregnancy outcomes and birth defects (2–4). Until now, the unresolved issues of the effects of military service in Vietnam or of exposure to dioxin-containing phenoxy-herbicides such as Agent Orange on male fertility have not been adequately studied (5–7).

In 1985 and 1986 the Centers for Disease Control (CDC) conducted a congressionally mandated health study of Vietnam veterans called the Vietnam Experience Study (VES). A summary of the physical health findings has been reported elsewhere (8). As part of the medical examinations, a sample of study participants underwent an evaluation of their semen characteristics. In this report we present detailed results on the semen characteristics of 324 Vietnam veterans compared with a similar group of 247 army veterans who did not serve in Vietnam.

METHODS

Participant selection

Study participants were selected from a random sample of enlisted male U.S. Army veterans who served during the Vietnam era. The current health evaluation consisted of a telephone health interview and a comprehensive medical and psychological examination of a random subsample of telephone interview participants. Of those selected, 2490 Vietnam veterans (75%) and 1972 non-Vietnam (63%) veterans participated in the examinations. All examinations were performed at a medical clinic in Albuquerque, New Mexico. Examination personnel and laboratory technicians did not know if

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participants had served in Vietnam.

In the initial VES protocol, semen analysis was not part of the medical examinations. However, early analyses of responses in the telephone interviews showed that Vietnam veterans were reporting more difficulty conceiving children. As a result, analysis of semen samples was added to the medical examination during the last 5 months of the 16-month period during which examinations were conducted. Of 705 veterans without vasectomy who were examined during this period, 571 (81%) participated. Participation rates were the same in the Vietnam and non-Vietnam groups. The characteristics of the semen analysis participants were similar to the entire group of medical examination participants.

Standardized protocols were followed for the collection and processing of semen samples. Participants collected samples in their hotel rooms. After voluntarily abstaining for at least 48 hours from any sexual activity that involved ejaculation, each participant collected a semen specimen by masturbating (without using lubricants or condoms) into a plastic container. The participant then placed the container in an insulated cup and, within 30 minutes of the collection, delivered the cup to the sample processing room in the hotel. The samples were stored in an incubator at 30 °C until they were liquefied. For video recording, 5 μL of the specimen was added to a Makler chamber and placed on a microscope viewing stage that had been warmed to 37 °C. Then, 30-second recordings were made of 8 different fields from preassigned areas of the chamber.

Semen analysis was performed with the Cellsoft computer-assisted semen analyzer system (9). This system has two major software components, a motility module and a morphology module. Using the motility module, we measured sperm concentration, percentage of motile sperm cells, linear velocity, straight-line velocity, linearity of motion, amplitude of lateral head displacement, and beat frequency.

For morphology and morphometry analysis, a drop of semen was placed onto a microslide, fixed in 95% ethanol,and stained with Papanicolaou (Pap) stain. The microslide was placed under a microscope, and each sperm head image was individually computer-analyzed for quantitative measurements and for classification of sperm head shape. The classification of sperm cell morphology was based on quantitative (morphometric) measurements of sperm head area, perimeter, length/width ratio, and major axis length. The five morphologic cell categories, based on World Health Organization criteria (10), were normal, large, small, tapered, and amorphous. Amorphous types included sperm with cytoplasmic droplets, abnormally shaped single heads, or double heads. The computer system could not analyze immature sperm or sperm with midpiece or tail abnormalities, so these types of sperm were not analyzed morphologically.

Table 1. Software parameter settings for measurement of sperm motility on videotapes, by microscope magnification

	Ocular magnification	
	× 1.0	× 1.5
No. of frames to analyze	15	15
No of frames per second	30	30
Video standard (A/E)	A	A
Minimum sampling		
Motile	1	1
Velocity	4	4
Maximum velocity, μm/sec	140	140
Threshold velocity, μm/sec	10	10
Threshold grey level	. . .[a]	. . .[a]
Cell color	White	White
Pixel scale, μm/pixel	0.688	0.459
Dilution factor	1.000	1.000
Cell size range, pixels	5–25	11–56
Lateral head displacement		
Minimum number of points	7	7
Minimum velocity, μm/sec	18	18
Minimum linearity	3.50	3.50

[a]Set at the beginning of each analytical run.

Some of the 571 participants specimen could not be analyzed for all of the semen characteristics. Three of the veterans' specimens contained no sperm. Several samples were lost because of technical difficulties during the processing procedures. Thus, microslide preparations from 566 specimens were analyzed for sperm morphology and morphometry, and 546 samples were video recorded for sperm concentration and motility analysis.

A majority of specimens were inadvertently videotaped at a higher magnification (× 1.5 versus × 1.0 for the ocular lens setting) than the computer software manufacturer recommended. As a result, the parameter settings for motility measurements were adjusted to account for differences in magnification. The settings used to analyze videotapes made at the two microscope magnifications are shown in Table 1.

We implemented several quality control procedures to ensure that all technicians used similar analytical methods throughout the study. For quality control of concentration and motility measurements, three videotapes (recorded at the × 1.0 ocular lens setting) were made of specimens with high, medium, and low-normal concentrations of sperm, respectively. These tapes were played at the beginning of each analytical run. Before the participants' specimens could be analyzed, the results from all three quality control tapes had to be within predetermined acceptable performance limits for sperm concentration, percentage of motile sperm, and mean linear velocity. For quality control of morphologic and morphometric measurements, a set of 20 microslides, prepared from a single specimen, was used. At the beginning of the study, all 20 slides were analyzed to determine acceptable performance limits for measure-

Table 2. Selected characteristics of Vietnam and non-Vietnam veterans undergoing semen analysis

Characteristic	Vietnam (n = 324) %	Non-Vietnam (n = 247) %
Race		
White	81	81
Black	14	12
Other	5	7
Age at examination (years)		
30–34	4	10
35–39	67	61
≥40	29	29
Current smoking status		
Nonsmoker	26	26
Ex-smoker	27	29
Current smoker	46	45
Illicit drug use (past year)		
None	79	76
Marijuana only	11	14
Other	10	10
Alcohol consumption (Avg. Drinks/Mo.)		
≤29	63	62
30–89	26	30
≥90	11	8
Marital Status		
Never married	10	8
Ever married	90	92
Education (Years)		
0–11	13	7
12–15	67	73
≥16	20	20
Family income (dollars)		
≤20,000	28	28
20,001–40,000	51	51
>40,000	21	21

ments of mean cell area, mean cell perimeter, mean length/width ratio, and mean major axis length. During the study, one slide (randomly chosen by the quality control supervisor) was analyzed at the start of each analytical run. Before participants' specimens could be analyzed, the quality control results had to be within the performance limits for each measure. In general, laboratory performance was good. The coefficients of variation (CVs), as measures of reliability, were ≤ 10% for all but one measure — the CV for the percentage of morphologically normal cells was 11.1%.

Differences between Vietnam and non-Vietnam veterans were consistently similar between technicians. Adjustment of Vietnam–non-Vietnam comparisons for technician did not alter the results.

Statistical methods

For statistical analysis of the data, we made several adjustments for technical factors associated with semen collection and processing. We excluded the motility data

Table 3. Reported fertility histories of Vietnam and Non-Vietnam veterans undergoing semen analysis

	Vietnam (n = 324) %	Non-Vietnam (n = 247) %
Infertility condition diagnosed by a doctor		
No	94	98
Yes	6	2
Failure to conceive with 1 partner, ≥ 1 year		
No	81	86
Yes	19	14
Total number of live births conceived after assignment to primary tour of duty		
0	31	25
1	24	25
2	26	30
≥ 3	19	20

on the 18 specimens (from 14 Vietnam and 4 non-Vietnam veterans) for which the time lapse between collection and videotaping was greater than 2 h and 20 min (or for which the time lapse could not be determined). For 63 specimens (from 37 Vietnam and 26 non-Vietnam veterans), the participants indicated that they had spilled some of the specimen as they were collecting it. These specimens were excluded from analyses of sperm concentration and number of cells per ejaculate. Within each specimen, individual sperm initially classified as "motile" but which had a linearity ≤ 1.0 or a straight-line velocity ≤ 5.0 μm/sec were reclassified as "nonmotile" for the statistical analyses and were excluded from computations of mean linear velocity, mean straight-line velocity, mean linearity, mean amplitude lateral-head displacement, and mean beat frequency. These types of cells accounted for < 2% of all motile cells with velocity measurements.

We used multiple linear regression (11) to evaluate differences in means between the two cohorts for the measures of sperm concentration, movement characteristics, morphology, and morphometry. Test results that were logarithmically normally distributed were transformed before analysis.

In addition to comparing mean values, we performed more detailed analyses on three measures of sperm quality: concentration, percentage of motile sperm, and percentage of sperm cells with "normal" morphology. These measures have been used the longest clinically, and more is known about their relationship to fertility potential than is known about other measures (10, 12–13). Major reductions in these measures (a concentration of ≤ 20 million sperm per milliliter of semen, a percentage of motile sperm < 40%, and a percentage of morphologically normal cells of < 40%) have traditionally been used as indicators of reduced fertility potential.

We used logistic regression (14) to evaluate Viet-

Table 4. Means and differences between means for measures of sperm counts and sperm movement characteristics among Vietnam and Non-Vietnam veterans

	Crude Mean[a]		Crude Results		Multivariate Results: Model 1[b]		Multivariate Results: Model 2[c]	
Measure	Vietnam	Non-Vietnam	Mean diff	95% CI	Mean diff	95% CI	Mean Diff	95% CI
Sperm counts								
Concentration, million cells/mL	64.8	79.8	−18.8*	−32.7, −2.1	−20.2*	−34.5, −2.8	−20.7*	−33.4, −5.4
Total count, million cells/ejaculate	146.7	184.7	−20.6*	−36.3, −1.1	−16.8	−33.9, 4.7	−16.7	−31.8, 1.8
Movement characteristics								
Motile cells, %	56.9	60.4	−3.4	−7.5, 0.6	−3.3	−7.6, 1.1	−2.1	−6.4, 2.2
Mean linear velocity, μm/sec	49.9	50.6	−0.7	−2.5, 1.0	−0.6	−2.5, 1.3	−0.1	−2.0, 1.8
Mean striaght line velocity, μm/sec	35.6	36.3	−0.7	−2.3, 0.9	−0.5	−2.3, 1.2	−0.1	−1.9, 1.6
Mean linearity	6.9	6.9	−0.0	−0.2, 0.1	−0.0	−0.2, 0.1	−0.0	−0.2, 0.2
Mean amplitude lateral head displacement, μm	2.1	2.1	−0.0	−0.1, 0.1	0.0	−0.1, 0.1	0.0	−0.1, 0.1
Mean beat frequency, Hz	14.9	14.9	−0.0	−0.4, 0.3	−0.0	−0.4, 0.3	−0.0	−0.4, 0.3

[a]Means and differences are arithmetic for all measures except concentration and total count; for these two measures, means are geometric, and mean difference refers to the percent difference in geometric means.
[b]Model 1 adjusts for year of entry into service, age at entry, enlistment status (drafted/volunteered), enlistment general technical score, military occupational specialty, and race.
[c]Model 2 adjusts for smoking status, illicit drug use, alcohol consumption, abstinence from sexual activity (Numer of days before sample collection), time between sample collection and analysis (in minutes), and videotaping magnification, in addition to the 6 covariates in Model 1.
*$P < 0.05$.

nam veterans' risk of having abnormalities in these three measures relative to non-Vietnam veterans. The measure of association computed in logistic regression is the odds ratio (OR). The OR is roughly equivalent to the ratio of prevalences of a condition (or abnormality) in the Vietnam veterans relative to the non-Vietnam veterans.

In both the multiple linear regression and logistic regression analyses two models were used. The first model (Model 1) included (and thus adjusted for) six variables in addition to cohort status: race, age at entry into the army, year of entry, type of enlistment (drafted or volunteered), enlistment general technical test score (a

Table 5. Means and differences between means for morphologic and morphometric sperm measures among Vietnam and Non-Vietnam veterans

	Crude Mean[a]		Crude Results		Multivariate Results: Model 1[b]		Multivariate Results: Model 2[c]	
Measure	Vietnam	Non-Vietnam	Mean Diff[d]	95% CI	Mean Diff[d]	95% CI	Mean Diff[d]	95% CI
Morphology								
Normal cells, %	57.9	60.8	−2.9*	−5.6, −0.2	−3.8*	−6.6, −0.9	−3.4*	−6.3, −0.5
Morphometry								
Mean cell area, μm^2	9.0	8.8	1.8	0.0, 3.8	1.4	−0.6, 3.5	1.4	−0.6, 3.4
Mean cell perimeter, μm	12.4	12.2	1.5*	0.4, 2.6	1.3*	0.2, 2.5	1.3*	0.2, 2.5
Mean cell length/ width ratio	1.7	1.6	1.6	−0.2, 3.5	1.6	−0.4, 3.6	1.6	−0.4, 3.5
Mean cell major axis length, μm	4.4	4.3	1.7*	0.4, 3.1	1.5*	0.1, 3.0	1.5*	0.1, 2.9

[a]The mean for the percentage of normal cells is arithmetic; all other means are geometric.
[b]Model 1 adjusts for year of entry into service, age at entry, enlistment status (drafted/volunteered), enlistment general technical score, military occupational specialty, and race.
[c]Model 2 adjusts for smoking status, illicit drug use, alcohol consumption, and abstinence from sexual activity (number of days before sample collection), in addition to the 6 covariates in Model 1.
[d]For the percentage of normal cells, the mean difference is arithmetic; for all other measures it is the percent difference in geometric means.
*$P < 0.05$.

Table 6. Prevalence of selected semen abnormalities among Vietnam and non-Vietnam veterans

Semen Abnormality	Vietnam		Non-Vietnam		Crude Results		Multivariate Results: Model 1[a]		Multivariate Results: Model 2[b]	
	%	N	%	N	OR	95% CI	OR	95% CI	OR	95% CI
Concentration ≤20 million cells/mL	15.9	42	8.1	17	2.1*	1.2–3.9	2.3*	1.2–4.3	2.7*	1.3–5.7
Motile cells <40%	28.0	83	23.4	54	1.3	0.9–1.9	1.2	0.8–1.8	1.1	0.7–1.7
Normal cells <40%	15.9	51	11.4	28	1.5	0.9–2.4	1.6	0.9–2.8	1.6	0.9–2.9

[a]Model 1 adjusts for year of entry into service, age at entry, enlistment status (drafted/volunteered), enlistment general technical score, military occupational speciality, and race.
[b]Model 2, in addition to the 6 covariates in Model 1, also adjusts for smoking status, illicit drug use, alcohol consumption, abstinence from sexual activity (number of days before sample collection), time between sample collection and analysis (in minutes), and taping magnification. The time and magnification variables were not included in the analysis of normal cells.
*$P < 0.05$.

measure of mental aptitude), and military occupational speciality (tactical or non-tactical). The second model (Model 2) was more comprehensive and, in addition to the six variables in Model 1, also included current smoking status, alcohol consumption, illicit drug use, and the number of days from the last previous ejaculation until the day the semen was collected. For sperm counts and movement characteristics, the time (in minutes) between semen collection and videotaping was also included as a covariate. An indicator variable for ocular magnification (× 1.5 versus × 1.0) during videotaping was included as a covariate for analyzing concentration, number of sperm cells per ejaculate, and sperm motility measures.

Additional variables were evaluated in regression analyses, but because they had little or no effect upon the comparison of cohorts, these covariates were not included in final regression models. These variables were income; education; drug use while in the army; use of antimalarial drugs in the Army; age at examination; region of birth; marital status; fathering no live births since entry into the army; body mass index; depression or anxiety in the past year; post-traumatic stress disorder (PTSD) in the past year; low and high levels of testosterone, of follicle-stimulating hormone, and of luteinizing hormone; urinary tract infection; prostatis; epididymitis; varicocele; gonorrhea; syphilis; genital herpes; malaria; month of semen collection; and current medication use (of those drugs that influence sperm production).

RESULTS

Among the semen analysis participants, the demographic, socioeconomic, and behavioral characteristics of the Vietnam and non-Vietnam veterans were similar (Table 2). The Vietnam veterans reported having more difficulties conceiving pregnancies and more physician-diagnosed infertility conditions than non-Vietnam veterans (Table 3). The proportion of veterans who had not fathered any children after entry into the army, however, was not significantly different between the two groups (31% versus 25%). The distribution of number of children fathered after entry into the army was similar in the two groups; the average number of live births fathered per veteran was 1.4 for the Vietnam veterans and 1.5 for the non-Vietnam veterans.

From the motility module measurements, we found that mean sperm concentration was 20% lower forVietnam veterans than for non-Vietnam veterans (64.8 versus 79.8 million cells/mL semen) (Table 4). A similar 20% decrease was seen in the mean number of sperm cells per ejaculate, but this difference diminished and was not statistically significant after adjustment for other characteristics. The mean proportion of motile sperm and other sperm motility measures were, on the average, similar for Vietnam and non-Vietnam veterans.

Results of morphology module measurements indicated that Vietnam veterans had a lower mean proportion of morphologically "normal" sperm than did non-Vietnam veterans (57.9% versus 60.8%) (Table 5). The Vietnam veterans tended to have slightly higher proportions of large, tapered, and amorphous sperm cells. Results of our analysis of morphometric data also indicated that specimens from Vietnam veterans were more likely to contain larger and more tapered sperm (Table 5). Both the mean cell perimeter and the mean length of the major axis of the cell were significantly larger for Vietnam than for non-Vietnam veterans. Regression analyses suggested that these cohort differences in morphometric measures were primarily attributable to differences among those who entered military service between 1970–71. However, the association between mean percentage of normal cells (a proportion that is based on a combination of these morphometric measures) and place of service was not restricted to these 2 years of entry.

Specimens from Vietnam veterans were twice as likely as specimens from non-Vietnam veterans (15.9% versus 8.1%) to have sperm concentrations less than or equal to the clinical reference value of 20 million

Table 7. Percent and number of Vietnam and non-Vietnam veterans with single and multiple types of semen abnormalities

	Vietnam		Non-Vietnam	
	%	N	%	N
Single Abnormality				
Concentration ≤ 20 million cells/mL	2.3	6	0.5	1
Motile cells < 40%	13.6	35	13.7	28
Normal class cells < 40%	6.2	16	5.4	11
Multiple Abnormalities				
Concentration ≤ 20 million cells/mL and motile cells < 40%	3.9	11	4.0	9
Concentration ≤ 20 million cells/mL and normal cells < 40%	1.3	4	0.0	0
Motile cells < 40% and normal cells < 40%	1.9	6	2.1	5
Concentration ≤ 20 million cells/mL and normal cells <40% and motile cells < 40%	5.1	16	1.7	4

cells/mL semen (Table 6). Differences in the proportion of men with semen specimens that contained less than 40% motile cells or less than 40% morphologically normal cells were not statistically significant. However, specimens from more Vietnam (5.1%) than non-Vietnam (1.7%) veterans had all three of these abnormalities (crude OR = 3.2, 95% CI 1.1–9.6) (Table 7).

Among Vietnam veterans, there were no trends in the percentage of veterans with semen abnormalties by level of self-reported combat or herbicide exposure (Table 8). The percentage of veterans with sperm abnormalities was less for those who reported taking antimalarial drugs during Vietnam service than for those who reported not taking these drugs.

DISCUSSION

Among the subsample of men whose semen characteristics were evaluated in the VES medical examinations, there were cohort differences in semen quality. In particular, results for Vietnam veterans showed about a 20% decrease in average sperm concentration, and twice the proportion of men with sperm concentrations at or below the clinical reference value of 20 million cells/mL semen. These cohort differences in sperm concentration were also reflected in similar decreases in the total number of sperm cells per ejaculate. In addition, results for Vietnam veterans showed a significantly lower average proportion of morphologically "normal" sperm heads. Measurements of the dimensions of sperm heads indicated that sperm from Vietnam veterans tended to have larger and more tapered heads than sperm from non-Vietnam veterans.

Other measures of semen characteristics and reproductive function were similar between the two cohorts. Measures of sperm movement, including the percentage of motile cells, velocity, linearity of motion, and lateral head motion, were alike in the two cohorts. As we have previously reported (8), testosterone, luteinizing hormone, and follicle stimulating hormone levels were similar in the Vietnam and non-Vietnam cohorts.

We do not believe that the differences between cohorts in sperm concentration and sperm head morphology are due to biases in study design or execution. Participation bias is unlikely, since both cohorts had similarly high (over 80%) participation rates in the semen analysis component.

Neither do we believe that the differences are due to information bias. The semen measurements were almost totally automated, with a computer making the measurements. In addition, the technicians who prepared the videotapes and slides of the semen specimens and who ran the computer analyses were not aware of the partic-

Table 8. Prevalence of selected semen abnormalities among Vietnam Veterans by self-reported army service experiences

	Type of Abnormality					
	Concentration ≤ 20 Million Cells/mL		Normal Cells < 40%		Motile Cells < 40%	
Experience	%	N	%	N	%	N
Combat exposure index[a]						
1 (Lowest)	15.3	9	12.5	9	30.8	21
2	15.9	10	17.6	13	27.1	19
3	15.0	12	17.9	17	27.6	24
4 (Highest)	18.0	11	15.2	12	25.4	18
Herbicide exposure[b]						
None	15.5	19	16.1	26	31.3	45
Low	15.3	13	15.6	15	21.7	20
Mid-High	18.2	10	16.1	10	28.3	17
Antimalarial drug use						
No	20.5	9	17.3	9	32.0	16
Yes	13.9	28	14.8	36	25.3	57

[a]Quartiles of reported frequency of combat experiences.
[b]Low = Walked through defoliated area.
Mid–High = Present during spraying, got herbicides on skin, sprayed herbicides, or handled herbicide equipment.

ipants' cohort status.

Confounding by other factors that could influence semen characteristics is also unlikely. For most of the important factors that could affect semen characteristics, such as race, age, and alcohol use, the two cohorts were similar. In addition, we performed multivariate analyses that adjusted for many personal variables (and for technical factors related to specimen collection and processing) that could have affected semen characteristics.

Since semen analysis was performed for only a subsample of the VES participants, questions may arise as to how representative the subsample is of all the VES participants and whether the findings may relate only to this particular subsample. The characteristics of the men who participated in the semen analysis were nearly identical to those of the entire group that participated in the medical examination. This suggests that the semen analysis participants were representative of the medical examination participants. The possibility that the findings in the semen analysis may have been spurious (or caused by chance) cannot be ruled out, but the level of significance of the statistical tests and the consistency in the differences between cohorts indicate that this explanation is unlikely.

The effects of the lower concentration of sperm and the fewer normal sperm cells upon reproductive function are difficult to determine. The exact correspondence between particular deficiencies in semen quality and fertility potential is not well established. Though major reductions in sperm quantity and quality are generally agreed to be associated with reduced fertility (15–18), the effects of subtle sperm changes on fertility remain uncertain (19–20). Various investigators have found that different aspects of semen quality may be best correlated with fertility. Some have found that two measures, sperm concentration and the percentage of normal sperm, correlate best with fertility (21). Others claim that motility measures correlate better (19, 20, 22–25). Male infertility, however, has many causes, and all of the factors that contribute to this condition are not likely to be reflected in a single test result or measure (26).,

Another reason why it is difficult to relate semen characteristics to fertility is that, in the past, the measurements of semen quality have been subject to variability and subjectivity. Only recently, with the technical development of computer-assisted semen analysis systems, has it become possible to obtain standardized, objective, and reproducible measures of semen characteristics (27). This new technology has also permitted investigators to measure certain semen characteristics, such as head dimensions, velocity, and lateral head motion, that previously could not be measured on a large scale. Because the technical developments are so recent, relatively little information is available on the relationship between these newer measures and fertility potential.

In clinical evaluations and epidemiologic studies, the measures that have been in use the longest include sperm concentration, sperm morphology, and the percentage of motile sperm (28). Major reductions in these measures have traditionally been used as indicators of reduced fertility potential (10, 12, 28–30). On the basis of certain clinical criteria (10, 12, 13), the Vietnam veterans had double the proportion of men with low sperm concentration ($\leq$ 20 million/mL) and about a 50% increase in the proportion of men with low levels ($<$ 40%) of normal sperm heads. There was also a significant decrease in the mean percentage of normal sperm heads for the Vietnam group. The two cohorts differed only slightly in the proportion of men with semen samples of low motility ($<$ 40% motile sperm cells). The proportion of men who had low values on all three measures was significantly higher for Vietnam veterans than for non-Vietnam veterans (5.1% versus 1.7%).

Another difficulty in trying to draw definitive conclusions from the semen differences noted in this study is that we evaluated current semen characteristics, whereas the participants had served in the Army 10 to 20 years before the study. We do not know how the semen characteristics for these men men may have changed in the intervening years. For example, the differences noted for the Vietnam veterans may have been stable over time or greater during and immediately after active duty, with a lessening over the years between that duty and this study. Results of studies of men exposed to dibromochloropropane (31,32) and radiation (33) have shown that, after exposure, semen quality initially decreases but then improves with increased time, provided the stem cells (type A spermatogonia) have not been destroyed.

The fertility histories of Vietnam and non-Vietnam veterans suggest that cohort differences in semen characteristics have not increased the number of Vietnam veterans unable to father children. Although Vietnam veterans reported more difficulties conceiving pregnancies, there were no significant differences between Vietnam and non-Vietnam veterans in the average number of children fathered after the men were assigned to their primary tour of duty (1.4 versus 1.5) or in the proportion who have not fathered any children (31% versus 25%).

These results — that Vietnam veterans reported more difficulties conceiving pregnancies and were more likely to have certain deficiencies in semen quality —are not necessarily incompatible with our finding that men in both cohorts fathered similar numbers of children. In a study in which investigators evaluated fertility over a 20-year period, low sperm counts were found to be related to the time interval to pregnancy, but pregnancy rates were not significantly affected unless the sperm count was below 5 million cells/mL semen (34). Thus, Vietnam veterans may have had a more difficult time

conceiving pregnancies, perhaps resulting in delays in conceiving pregnancies, but, overall, Vietnam and non-Vietnam veterans have been able to father similar numbers of children.

Less clear are the implications of the observed differences in semen characteristics for pregnancy outcomes. Results of some investigations in animals, mainly mice, suggest a relationship between induced sperm changes and heritable genetic damage. However, no results of studies among humans have clearly shown that sperm head changes are related to adverse reproductive outcomes (19) or to birth defects. Results of early studies and case reports suggested that poor semen quality was associated with spontaneous abortions and other adverse pregnancy outcomes (35, 36). In a more recent study, however, investigators found no evidence that diminished semen quality is responsible for spontaneous abortions (37). In the VES, children of Vietnam veterans and non-Vietnam veterans had similar rates of documented birth defects (38).

We could not determine the reasons for the differences in sperm characteristics between Vietnam and non-Vietnam veterans. The VES was designed to evaluate health effects resulting from the general experience of military service in Vietnam. Little information, mostly from the participants' recollections, was available regarding the specific components that may have made up the Vietnam service experience.

Possible explanations for the semen findings in the Vietnam cohort include exposure to infectious agents (28), illicit drug use (39), alcohol use (40–42), and the general stress (43) of serving in a combat situation. We evaluated the influence of reported current use of alcohol, marijuana, other illicit drugs, cigarettes, and certain medications and found that they did not account for the differences in sperm characteristics between Vietnam and non-Vietnam veterans. Neither did we find that the more prevalent psychological disturbances experienced by Vietnam veterans, including anxiety, depression, alcohol abuse or dependence, and post-traumatic stress disorder, accounted for the differences. Neither were past sexually transmitted diseases, as reported by the veterans, related to the sperm differences between cohorts, nor were the differences accounted for by technical factors, such as time between last ejaculation and specimen collection or time between specimen collection and analysis.

The differences in sperm characteristics between the Vietnam and non-Vietnam cohorts did not appear to be specific to a particular subgroup of veterans. Although some of the morphometry results suggested that the findings of larger and more tapered sperm heads for the Vietnam group may have been restricted to those Vietnam veterans who entered the service in 1970–71, the analysis of sperm morphology classifications did not substantiate this conclusion, that is, having fewer normal sperm heads was not restricted to Vietnam veterans who entered the Army in 1970–71. Neither were the concentration differences limited to these 2 years of enlistment. In addition, the findings do not appear to be accounted for by reported use of heroin or other drugs (including illicit and antimalarial drugs) while in the Army. The level of combat experienced in Vietnam was not related to the semen findings, because there were no associations between abnormal semen characteristics and having either a tactical MOS or higher levels of self-reported combat experience.

The possibility that exposure to dioxin-containing herbicides may have affected the sperm of Vietnam veterans is a potential concern. However, this explanation seems unlikely for several reasons. First, no results of human studies have been reported that show reduced male fertility following exposure to dioxin. Evaluations of the reproductive effects of dioxin among humans, including semen analysis, are limited. An unpublished report has been cited (44) of railroad workers who, after cleaning up a dioxin-contaminated spill, had a 40% average decrease in sperm count and decreased plasma testosterone levels compared with unexposed workers. However, the report did not include data on which we could base an assessment of these workers' reproductive history. The U.S. Air Force Ranch Hand Study (4) evaluated reproductive function in a group of men potentially exposed to dioxin during Agent Orange spraying in Vietnam. The distributions of sperm concentration and of the percentages of abnormally shaped sperm were similar for the Ranch Hand and comparison groups. The group exposed to herbicide did not have more fertility problems, nor did the two groups differ in the mean number of conceptions per veteran or in the proportion of men with childless marriages.

Another reason the semen results are unlikely to be due to dioxin exposure is that in a recent study we found that few Army ground troops were heavily exposed to dioxin-containing herbicides (45). Furthermore, in the VES, we found that semen characteristics did not differ according to levels of self-reported herbicide exposure.

In conclusion, 10 to 20 years after military service in Vietnam, certain differences in semen characteristics are evident in Vietnam veterans relative to a comparable group of veterans who did not serve in Vietnam. The largest difference is a decrease in the concentration of sperm; another significant difference is a decrease in the average proportion of normal sperm cells. These differences in sperm quality may have caused some difficulties in Vietnam veterans' ability to conceive pregnancies, but they do not appear to have increased the proportion of Vietnam veterans who have been unable to father children.

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REFERENCES

1. Bogen, G. Symptoms in Vietnam veterans exposed to Agent Orange (letter to the editor). JAMA. 1979; 242:2391.
2. Donovan JW, Adena MA, Rose G, Batistutta D. Case-control study of congenital anomalies and Vietnam service. Canberra, Australia: Government Publishing Services, 1983.
3. Erickson JD, Mulinaire J, McClain PW, *et al.* Vietnam veterans' risks for fathering babies with birth defects. JAMA. 1984: 252:903–12.
4. Lathrop GD, Wolfe WH, Albanese RA, Moynahan PM. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides; baseline morbidity study results. Brooks Air Force Base, Texas: U.S. Air Force School of Aerospace Medicine: 1984.
5. Lamb JC, Moore JA. Effects of phenoxy acid herbicides and TCDD on male reproduction. In: Lobl TJ, Hafez ESE, eds. Male fertility and its regulation. Boston: MTP Press Limited; 1984: 269–86.
6. Mattison DR, Nightingale MS, Silbergeld EK. Reproductive toxicity of tetrachlorodibenzo-p-dioxin. In: Lowrance WW, ed. Public health risks of the dioxins. Los Altos, CA: William Kaufman; 1984:217–43.
7. Schrag SD, Dixon RL. Occupational exposures associated with male reproductive dysfunction. Ann Rev Pharmacol Toxicol. 1985; 25:567–92.
8. Centers for Disease Control Vietnam Experience Study. Health status of Vietnam veterans: II. Physical health. JAMA. 1988; 259:2708–14.
9. Ast M, Kahn M, Rosenberg S. Cellsoft user manual. New York: CRYO Resources; 1986.
10. Belsey MA, Eliasson R, Gallegos AJ, Moghissi KS, Paulsen CA, Prasad MRN. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. Singapore: Press Concern; 1980:1–24.
11. Draper NR, Smith H. Applied regression analysis. 2nd ed. New York: John Wiley + Sons; 1981:218–50.
12. Alexander NJ. Male evaluation and semen. Clin Obstet Gynecol. 1982; 25:463–82.
13. Chong AP, Walters CA, Weinried SA. The neglected laboratory test: the semen analysis. J Androl. 1983; 4:280–2.
14. Engelman L. Stepwise logistic regression. In: Dixon WJ, Brown MB, Engelman L, et al. (eds). BMDP statistical software. Berkeley: University of California Press; 1983:330–44.
15. Meistrich ML, Brown CC. Estimation of the increased risk of human infertility from alterations in semen characteristics. Fertil Steril. 1983; 40:220–30.
16. Smith KD, Rodriguez–Rigau LJ, Steinberger E. Relation between indices of semen analysis and pregnancy rate in infertile couples. Fertil Steril. 1977; 28:1314–9.
17. Wickings EJ, Freischem CW, Langer K. Nieschlag E. Heterologous ovum penetration test and seminal parameters in fertile and infertile men. J Androl. 1983; 4:261–71.
18. Zukerman Z, Rodriguez–Rigau LJ, Smith KD, Steinberger E. Frequency distribution of sperm counts in fertile and infertile males. Fertil Steril. 1977; 28:1310–3.
19. Wyrobek AJ, Gordon LA, Watchmaker G, Moore DH II. Human sperm morphology testing: description of a reliable method and its statistical power. In: Bridges GA, Butterworth BE, Weinstein IB, eds. Indicators of genotoxic exposure. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1982:527–41.
20. Wyrobek AJ, Watchmaker G, Gordon L. An evaluation of sperm tests as indicators of germ-cell damage in men exposed to chemical or physical agents. In: Lockey JE, Lemasters GK, Keye WR, eds. Reproduction: the new frontier in occupational and environmental health research. New York: Alan R. Liss; 1984: 385–405.
21. Sherins RJ, Howards SS. Male infertility. In: Walsh PC, Gittes RF, Perlmutter AD, Stamey TA, eds. Campbell's urology. Philadelphia: WB Saunders: 1986:640–97.
22. Aitken RJ, Best FSM, Richardson DW, Djahanbakhch O, Lees MM. The correlates of fertilizing capacity in normal fertile men. Fertil Steril 1982; 38:68–76.
23. Aitken RJ, Best FSM, Richardson DW, Djahanbakhch O, Mortimer D, et al. An analysis of sperm function in cases of unexplained infertility: conventional criteria, movement characteristics, and fertilizing capacity. Fertil Steril. 1982; 38:212–21.
24. Aitken RJ, Sutton M, Warner P, Richardson DW. Relationship between the movement characteristics of human spermatozoa and their ability to penetrate cervical mucus and zona-free hamster oocytes. J Reprod Fertil. 1985; 73:441–9.
25. Collins JA. Diagnostic assessment of the infertile male partner. Curr Probl Obstet Gynecol Fertil. 1987; 10:173–224.
26. Albertsen PC, Chang TSK, Vindivich D, Robinson JC, Smyth JW. A critical method of evaluating tests for male infertility. J Urol. 1983; 130:467–74.
27. Katz DF, Davis RO. Automatic analysis of human sperm motion. J Androl. 1987; 8:170–81.
28. Wyrobek AJ, Gordon LA, Burkhart JG, et al. An evaluation of human sperm as indicators of chemically induced alterations of spermatogenic function. Mutat Res. 1983; 115:73–148.
29. MacLeod J, Gold RZ. The male factor in fertility and infertility. III. An analysis of motile activity in spermatozoa of 1000 fertile men and 1000 men in infertile marriage. Fertil Steril. 1951; 2:187–204.
30. MacLeod J, Gold RZ. The male factor in fertility and infertility. IV. Sperm morphology in fertile and infertile marriage. Fertil Steril. 1951:2:394–414.
31. Eaton M, Schenker M, Whorton MD, Samuels S, Perkins C, Overstreet J. Seven-year follow-up of workers exposed to 1,2-dibromo-3-chloropropane. J. Occup Med. 1986; 28:1145–50.
32. Potashnik G. A four-year reassessment of workers with dibromochloropropane-induced testicular dysfunction. Andrologia. 1983; 15:164–70.
33. Meistrich ML. Critical components of testicular function and sensitivity to disruption. Biol Reprod. 1986; 34:17–28.
34. Bostofte E, Serup J, Rebbe H. Relation between sperm count and semen volume, and pregnancies obtained during a twenty-year follow-up period. Int J Androl. 1982; 5:267–75.
35. Joel CA. New etiologic aspects of habitual abortion and infertility, with special reference to the male factor. Fertil Steril. 1966; 17:374–80.
36. MacLeod J, Gold RZ. The male factor in fertility and infertility. IX. Semen quality in relation to accidents of pregnancy. Fertil Steril. 1957; 8:36–49.
37. Homonnai ZT, Paz GF, Weiss JN, David MP. Relation between semen quality and fate of pregnancy: retrospective study on 534 pregnancies. Int J Androl. 1980; 2:574–84.
38. Centers for Disease Control Vietnam Experience Study. Health status of Vietnam veterans: III. Reproductive outcomes and child health. JAMA. 1988; 259:2715–9.
39. Singer R, Ben-Bassat M, Malik Z, et al. Oligozoospermia, asternozoospermia, and sperm abnormalities in ex-addicts to heroin, morphine, and hashish. Arch Androl. 1986; 16:167–74.
40. Brzek A. Alcohol and male fertility (preliminary report). Andrologia. 1987; 19:32–6.
41. Gallant DM. Cytological abnormalities in sperm of alcoholics. Alcoholism Clin Exp Res. 1986; 10:554–5.
42. Kucheria K, Saxena R, Mohan D. Semen analysis in alcoholic dependence syndrome. Andrologia. 1985; 17:558–63.
43. Poland ML, Giblin PT, Ager JW, Moghissi KS. Effect of stress on semen quality in semen donors. Int J Fertil. 1986; 31:229–31.
44. Fabro S, Brown NA, Scialli AR. Agent Orange and dioxin. Reprod Toxicol Med Lett. 1984; 3:5–7.
45. Centers for Disease Control Veterans Health Studies. Serum 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin levels in U.S. Army Vietnam-era veterans. JAMA. 1988; 260:1249–54.