

Sperm Studies in Anesthesiologists

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Semen samples were collected from 46 anesthesiologists each of whom had worked a minimum of one year in hospital operating rooms ventilated with modern gas-scavenging devices. Samples collected from 26 beginning residents in anesthesiology served as controls. Concentrations of sperm and percentages of sperm having abnormal head shapes were determined for each sample. No significant differences were found between anesthesiologists and beginning residents. Limiting the analyses to men having no confounding factors (varicocele, recent illness, medications, heavy smoking, frequent sauna use) did not change the results. The sperm concentration and morphology in 13 men did not change significantly after one year of exposure to anesthetic gases. However, the group of men who had one or more confounding factors (excluding exposure to anesthetic gases) showed significantly higher percentages of sperm abnormalities than did the group of men without such factors. These results suggest that limited exposure to anesthetic gases does not significantly affect sperm production as judged by changes in sperm concentration and morphology. These data are reassuring, but since the hospitals surveyed used modern gas-scavenging devices, men who are occupationally exposed to anesthetic gases without this protection should be studied for fuller assessment of the possible human spermatotoxic effects. (Key words: Anesthesiologists. Anesthetics, gases: trace concentrations. Operating rooms: scavenging. Human spermatogenesis; sperm morphology. Toxicity: mutagenicity; spermatotoxicity.)

IN RECENT EPIDEMIOLOGICAL STUDIES, significant increases in spontaneous abortion rates and congenital abnormalities were found among the live-born children of anesthesiologists, nurse anesthetists, and other operating room personnel occupationally exposed to trace amounts of anesthetic gases.¹⁻⁸ Similar results were seen in dentists

and dental assistants who used inhalational anesthetics.⁹⁻¹⁰ Several studies have demonstrated elevated levels of spontaneous abortions and congenital abnormalities in the offspring of men occupationally exposed to inhalational anesthetics (mainly nitrous oxide and halothane), even when the wives were not exposed.^{1,4,6,7,9,10} However, the findings in unexposed wives are generally less marked and less consistent than those for women who are directly exposed to anesthetic gases.

Several animal studies have shown that exposure of males to anesthetic gases can lead to spermatogenic damage and induce chromosomal damage in the germ cell. Kripke *et al.*¹¹ exposed rats to 20 per cent nitrous oxide for up to 35 days. They found a reversible decrease in the number of testicular sperm and described "giant, multinucleated cells" in the seminiferous epithelium. Gremigni *et al.*¹² reported similar results in the testes of rats who were exposed to 20 per cent nitrous oxide for seven weeks. Coate *et al.*¹³ found dose-dependent increases in chromosomal aberrations in spermatogonial cells of rats exposed for 52 weeks to one ppm halothane and 50 ppm nitrous oxide or 10 ppm halothane and 500 ppm nitrous oxide.

Sperm studies in mice have shown that *in vivo* exposure to chemicals can cause dose-related increases in the proportion of abnormally shaped sperm that may indicate mutagenicity (see references 14 and 15 for a review and evaluation of the mouse sperm-morphology assay). Land *et al.*¹⁶ used this assay to survey the effects of eight different inhalational anesthetics and found that chloroform, trichloroethylene, and enflurane induced small but significant morphological changes in spermatozoa. These animal studies suggest that inhalational anesthetics may be spermatotoxic and cause genetic damage in mammals.

Changes in human sperm counts and sperm-shape abnormalities have also been demonstrated to be effective indicators of chemically induced spermatogenic damage.¹⁷ While the relationship of sperm defects to heritable effects in humans is not well understood, several studies (*e.g.*, references 18, 19) have suggested that reduced semen quality (*i.e.*, lowered sperm counts and increased levels of abnormally shaped sperm) may be related to an increased incidence of spontaneous abortion.

With these animal and human studies as background, we proposed to use human sperm count and morphology to assess the potential spermatotoxic effects of occupational exposure to anesthetic gases in a modern hospital setting.

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Methods

DESCRIPTION OF COHORT

Semen samples were collected from 46 anesthesiologists at three San Francisco Bay Area hospitals. This study was approved by the Human Subjects Committee of Stanford University and Lawrence Livermore National Laboratory, and informed written consent was obtained from each participant. Each man had been exposed for a minimum of one year to anesthetic gases in hospital operating rooms that used modern trace-gas-scavenging techniques. Twenty-six men were sampled before starting their anesthesiology residency and served as controls. One of these men was azoospermic so only 25 samples were available for analyses of sperm morphology. At the end of the first year of anesthesia residency, samples from 13 men were obtained and compared individually with samples obtained before exposure.

QUESTIONNAIRE

Detailed histories were taken on each participant including anesthetic exposure history (including number of hours per week in the operating room during that year and the total number of years working with anesthetic gases), personal habits (smoking, alcohol consumption, and sauna and hot tub use), and medical history (recent or major illnesses, urogenital tract infections, varicocele, medications, surgery, etc.). Several of these factors are suspected to affect human sperm production.²⁰⁻²³ These confounding factors were evaluated for their relationship to sperm concentration or morphology.

ESTIMATES OF EXPOSURE

The levels of nitrous oxide in the operating rooms of the three hospitals were monitored regularly. Sampling frequencies ranged from once-a-week to once-a-month. Trace amounts of nitrous oxide were present in all air samples taken during cases in which nitrous oxide was being used (mean < 50 ppm, range 5-300 ppm). When the rooms were not in use or when a regional anesthetic was given, the nitrous oxide levels were less than 5 ppm. These values were similar in the three hospitals. All operating rooms sampled had 12 to 17 air exchanges/h and had modern gas-scavenging devices. Scavenging consisted of the collection of waste gases from relief valves of all anesthesia machines and ventilators. Gases were vented to the outside by direct connection to the exhaust ducts of the non-recirculating air-conditioning systems.

To determine whether semen anomalies were related to exposure levels, 33 of the 46 exposed men (excluding the 13 repeat samples) were classified according to the average exposure in the operating room during the past year. Three men were exposed for less than 10 h/week,

14 men for 10 to 30h/week, and 16 men reported more than 30h/week of occupational exposure to inhalational anesthetics.

SEMEN ANALYSES

Semen samples were collected in clean glass containers after at least 24 h of abstinence. To assure a high participation rate we did not insist on a longer abstinence period. We asked donors to collect samples at home and bring them to the laboratory within 6 h. Since we could not control temperature and time since ejaculation, we did not assess sperm motility. Using a hemocytometer, sperm concentrations were determined from an aliquot of semen sample diluted 1:10 with phosphate-buffered saline containing 5 per cent formalin. Coded smears from the undiluted sample were prepared, air-dried, fixed, and stained using a modified Papanicolaou method as described elsewhere.²⁴ At least 500 sperm/sample were scored blind for sperm-head-shape abnormalities under a light microscope and classified into one of 10 shape categories (fig. 1). Periodic reevaluation utilizing a set of standard reference slides during the scoring process assured consistency of our scoring criteria. Furthermore, a comparison of 10 coded slides rescored after a one-year interval showed no change in our scoring criteria with time (mean \pm SEM, 45.4 \pm 4.3 per cent *vs.* 45.0 \pm 3.9 per cent).

STATISTICAL ANALYSIS

A variety of statistical tests were used to make comparisons between groups. The *t* test was used when data could be assumed to be normally distributed.²⁵ Non-parametric tests (*i.e.*, Mann-Whitney and Kolmogorov-Smirnov) were used when data were not distributed normally.^{25,26} Proportions were compared by the *z* test.²⁵ All tests were one-sided since we were looking for detrimental changes in sperm production.

Results

EFFECTS OF ANESTHETIC GASES

The distributions of the percentage of abnormally shaped sperm from exposed anesthesiologists and beginning residents (controls) did not differ (mean \pm SEM, 46.1 \pm 2.0 per cent *vs.* 47.7 \pm 2.5 per cent) (fig. 2, table 1). When men with confounding factors were excluded from the analysis, the difference remained statistically nonsignificant (46.4 \pm 3.6 per cent and 43.8 \pm 2.3 per cent, respectively) (table 1).

The sperm of occupationally exposed anesthesiologists were compared to determine whether their sperm anomalies were related to the total number of hours they worked in the operating room. No statistically significant

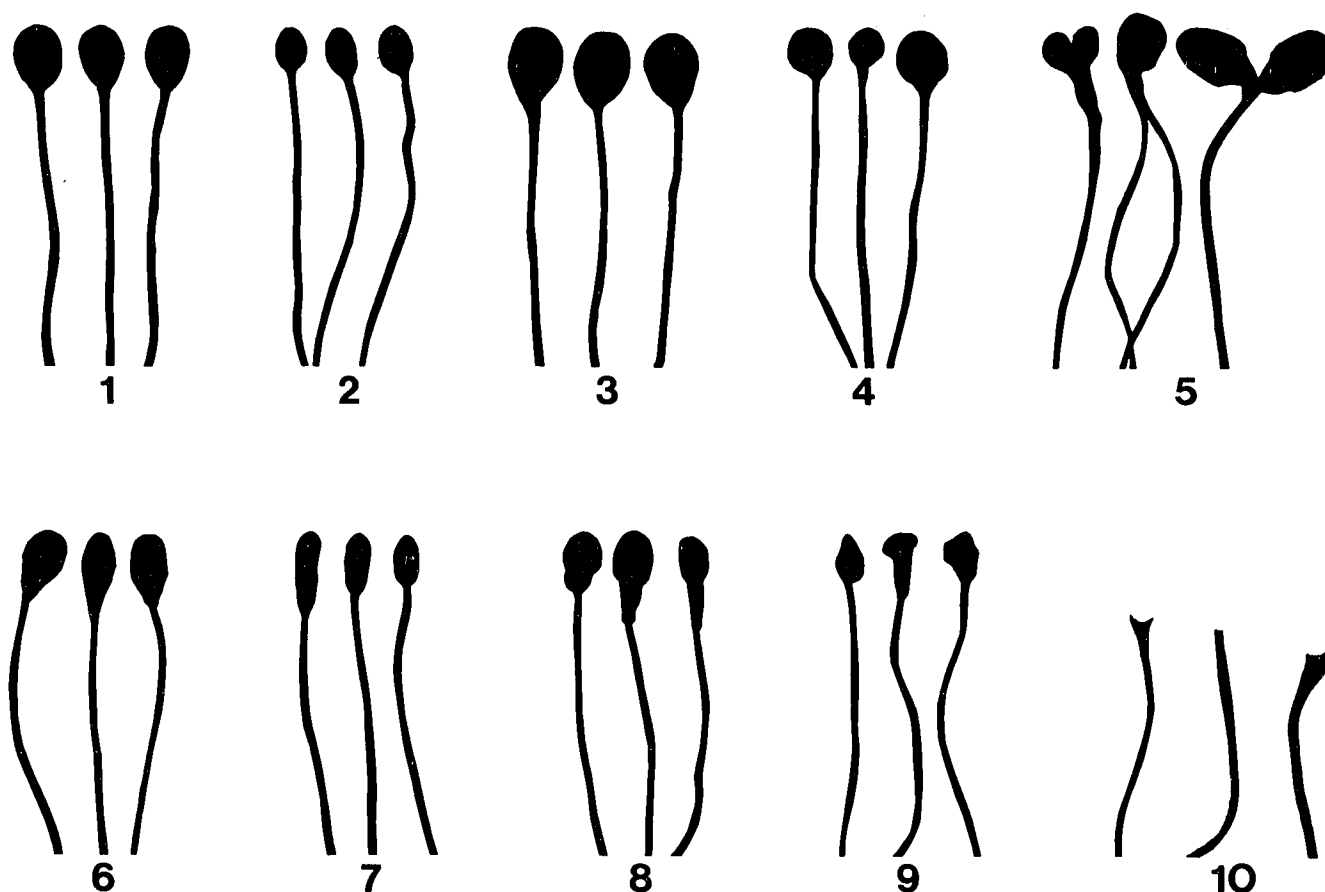


FIG. 1. Classes of human sperm-head shape. 1) Represents oval (normal) sperm. The classes of sperm shapes considered by us as abnormal are: 2) small, 3) large, 4) round, 5) double, 6) narrow-at-the-base, 7) narrow, 8) pear-shaped, 9) amorphous, and 10) ghost-like.

relationships were found between the average number of hours worked in an operating room and either sperm morphology (table 1) or sperm concentration (table 2).

Thirteen men were sampled twice, once before beginning work and again after one year of exposure as residents in anesthesiology (see table 3). Three of these individuals showed significant changes in abnormal sperm; in two cases the abnormal sperm decreased by 7 per cent ($P < 0.05$) and 15.1 per cent ($P < 0.001$), and in the third case the abnormal sperm increased by 14.2 per cent ($P < 0.001$). We were unable to identify possible causes for these changes from their responses to the questionnaire. Comparing the results from all 13 men we found no statistically significant changes in sperm morphology or concentration after the year of exposure to anesthetic gases (table 3).

EFFECT OF AGE AND CONFOUNDING FACTORS

Although the anesthesiologists were slightly older than the beginning residents (mean \pm SEM, 30.0 ± 0.4 years *vs.* 27.9 ± 0.5 years), this difference was not associated with a difference in sperm morphology. Furthermore, we saw no significant correlation between age and sperm

morphology when the data for anesthesiologists and residents were pooled.

The effects of confounding factors in the 72 samples were studied by dividing the data into two groups: those collected from men with, and those collected from men without confounding factors, irrespective of exposure. We considered five confounding factors: heavy cigarette smoking ($n = 1$), frequent sauna or hot tub use ($n = 6$), varicocele ($n = 3$), recent illness or urogenital tract infection ($n = 5$), current use of medications ($n = 5$), and combinations of the above ($n = 6$). These factors are suspected to affect human spermatogenesis.²⁰⁻²³ No subjects reported previous exposure to hazardous agents. The proportion of men with confounding factors in the control ($11/26 = 42.3$ per cent) and exposed populations ($15/46 = 32.6$ per cent) did not differ significantly. We found an overall increase in the percentage of abnormal sperm in those men having one or more confounding factors (50.1 ± 2.6 per cent, $n = 26$) compared to those not reporting any factors (44.6 ± 1.9 per cent, $n = 45$; $P = 0.05$). This difference is significant by both the *t* test and the Mann-Whitney test. A similar comparison of sperm counts did not detect any differences related to

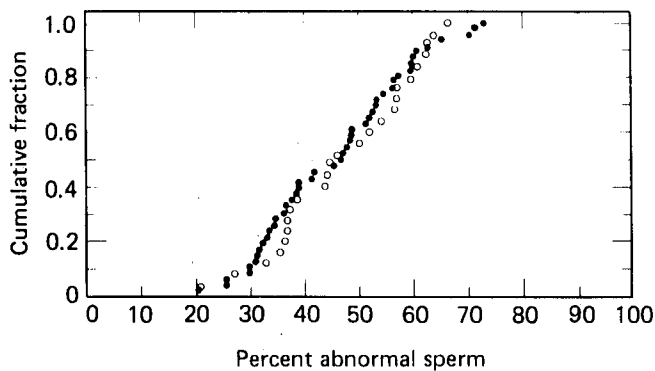


FIG. 2. Distribution of sperm morphology in anesthesiologist and residents unexposed to anesthetic gases. Each point represents the percentage of abnormal sperm from a single anesthesiologist (closed circle) or control subject (open circle), based on 500 sperm scored per sample. The data from the men in each group are ranked and plotted as a cumulative distribution.

confounding factors ($60.7 \pm 7.8 \times 10^6/\text{ml}$, $n = 46$ vs. $61.2 \pm 7.9 \times 10^6/\text{ml}$, $n = 26$). The sample sizes were too small to detect effects of specific confounding factors.

Discussion

A comparison of sperm counts and sperm shape abnormalities in the semen of 46 anesthesiologists and 26 control subjects showed no differences related to exposure to anesthetic gases. Detailed comparison of sperm from 13 men sampled before and after one year of exposure to anesthetic gases confirmed this finding. Although the sperm-abnormality assay was sufficiently sensitive to

detect an increase resulting from the confounding factors, the number of men with specific factors were too small to assess their relative impact.

The semen of the control subjects used in this study were compared with 48 historical controls consisting of 34 newly hired employees from a pesticide production plant in the Eastern USA²⁷ and 14 scientists in the Western USA (unpublished data). The beginning residents showed a statistically significant elevation in the percentage of abnormal sperm when compared to the historical control group, (47.7 ± 2.5 per cent vs. 42.4 ± 1.8 per cent, $P < 0.05$). In this case a Mann-Whitney two-tailed test was used since *a priori* we did not know which group would have a higher mean. We do not know the reason for this difference. A comparison of readings of the standard slides used during these analyses suggests that these differences did not result from differences in slide preparation or from variability in scoring criteria. This finding also appears not to be due to a difference in the proportion of men with confounding factors ($11/26 = 42.3$ per cent of the beginning residents vs. $16/48 = 33.3$ per cent of the historical controls, $P = 0.44$). The average age of the historical control group was not different from the beginning residents (mean \pm SEM, 28.9 ± 1.0 yr vs. 27.9 ± 0.5 yr).

We found that the average sperm concentration in the historical group was higher than in the beginning residents group ($116.5 \pm 18.0 \times 10^6/\text{ml}$ vs. $66.2 \pm 11.2 \times 10^6/\text{ml}$, $P < 0.05$, Cochran's *t* test). However, little should be made of this difference in counts because it may have resulted from the different abstinence period re-

TABLE 1. Sperm Morphology among Participants in Study

	Collection Date	Exposure Estimate	All Participants		Participating with Confounding Factors*		Participants without Confounding Factors	
			Sample Size	Per Cent†	Sample Size	Per Cent	Sample Size	Per Cent
Resident Controls	1978‡	none	14	49.5 ± 3.6	6	54.3 ± 4.0	8	45.9 ± 5.4
	1979	none	11	45.3 ± 3.6	5	43.4 ± 5.9	6	46.9 ± 4.8
Total for 1978 and 1979			25§	47.7 ± 2.5	11	49.4 ± 3.7	14	$46.4 \pm 3.6§$
Exposed Anesthesiologists	1978	<10 h/wk	3	56.2 ± 5.6	2	61.1	1	46.5
		10-30 h/wk	14	42.0 ± 2.8	6	46.2 ± 4.6	8	39.0 ± 3.2
		<30 h/wk	16	47.1 ± 3.7	4	50.7 ± 9.8	12	45.9 ± 4.0
Total for 1978			33	45.8 ± 2.3	12	50.2 ± 4.1	21	43.3 ± 2.7
	1979‡	NE¶	13	46.8 ± 4.1	3	52.8 ± 11.1	10	45.0 ± 4.4
Total for 1978 and 1979			46	46.1 ± 2.0	15	50.7 ± 3.7	31	43.8 ± 2.3

* The confounding factors represented were heavy smoking, frequent sauna use, varicocele, recent illness or urogenital tract infection, current use of major medications, and prior exposure to potentially hazardous agents.

† Mean percentage of abnormal sperm based on scoring 500 sperm/

sample \pm SEM.

‡ 1979 exposed group represents the same individuals as 1978 controls (minus one individual) after 1 year of exposure.

§ Does not include one man who was azoospermic.

¶ No exposure estimates were made.

TABLE 2. Sperm Concentrations among Participants in Study

	Collection Date	Exposure Estimate	All Participants		Participants with Confounding Factors*		Participants without Confounding Factors	
			Sample Size	Count†	Sample Size	Count	Sample Size	Count
Resident Controls	1978‡ 1979	none none	14	65.0 ± 15.5	6	50.5 ± 20.1	8	75.9 ± 22.8
Total for 1978 and 1979			13	67.6 ± 16.3	5	67.8 ± 20.2	7	67.4 ± 27.3
			26§	66.2 ± 11.2	11	58.4 ± 13.8	15	71.9 ± 16.9§
Exposed Anesthesiologists	1978	<10 h/wk 10-30 h/wk >30 h/wk	3	66.3 ± 17.0	2	76.5	1	46.3
Total for 1978			14	54.1 ± 8.8	6	68.0 ± 16.9	8	43.8 ± 7.8
			16	52.1 ± 7.8	4	68.0 ± 21.8	12	44.3 ± 8.1
Total for 1978 and 1979	1979‡	NE¶	33	53.3 ± 5.5	21	69.4 ± 10.8	12	44.1 ± 5.4
			13	69.3 ± 18.4	10	39.0 ± 20.0	3	78.4 ± 22.8
			46	57.9 ± 6.5	31	63.3 ± 9.8	15	55.2 ± 8.5

* See legend of Table 1 for list of confounding factors.
 † Mean sperm count (×10⁶/ml) ± SEM.
 ‡ 1979 exposed group represents the same individuals as 1978 con-

trols (minus one individual) after 1 year of exposure.
 § Includes one man who was azoospermic.
 ¶ No exposure estimates were made.

quirements for the two groups, *i.e.*, two to three days for the historical control group *vs.* one day for the new resident controls. In other studies, abstinence period has not affected sperm morphology.²⁸

The hospitals sampled in this study used modern gas-scavenging equipment. Previous reports demonstrating significant effects of paternal exposure on obstetric outcomes, particularly the dental surveys, were conducted among men working in operating rooms and dental operatories with higher concentrations of waste anesthetic gases. For example, ambient gas concentrations during

dental surgery in unscavenged rooms may exceed 73 ppm halothane and range from 500 to 6000 ppm nitrous oxide.⁹ These levels are 10 to 100 times higher than those found in the operating rooms of the hospitals that we surveyed. Although our present study indicates that no adverse effect on sperm counts or morphology occur in men working in scavenged operating rooms, the question of how semen quality is affected in men exposed to higher amounts of waste anesthetic gases remains unanswered. We believe that exposure to anesthetic gases in unscavenged or poorly scavenged operating rooms or dental surgeries should be studied to determine what effects, if any, exposure to higher concentrations of waste anesthetic gases have on seminal parameters.

TABLE 3. Sperm Morphology and Counts in Men Sampled before and after One Year of Exposure

Abnormal Sperm (Per Cent)			Sperm Count (× 10 ⁶ /ml)		
Before	After	Δ	Before	After	Δ
38.4	36.7	-1.7	169	43	-126
35.7	33.4	-2.3	31	27	-4
61.4	63.4	+2.0	65	39	-26
50.4	48.8	-1.6	19	138	+119
57.6	71.8	+14.2‡	34	12	-22
37.0	30.0	-7.0*	70	41	-29
52.3	52.4	+0.1	23	28	+5
63.0	47.9	-15.1‡	26	105	+79
37.0	34.2	-2.8	148	40	-108
21.2	20.6	-0.6	183	256	+73
60.4	60.6	+0.2	60	52	-8
57.4	55.2	-2.2	57	78	+21
54.6	53.2	+1.4	55	109	+54
MEAN 48.2	46.8	1.4‡	72.3	74.5	+2.2‡
SEM 3.6	1.4	1.8	15.8	18.4	19.4

* Analyses of proportion by z test,²¹ P < 0.05.
 † Analyses of proportion by z test,²¹ P < 0.001.
 ‡ By paired t test, ²¹ not significant.

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