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Methods for detecting occupational causes of male infertility

Reproductive history versus semen analysis

by Richard J Levine, MD¹

LEVINE RJ. Methods for detecting occupational causes of male infertility: Reproductive history versus semen analysis. *Scand j work environ health* 9 (1983) 371–376. The use of semen and reproductive histories in epidemiologic studies is reviewed. Traditional parameters of semen quality are easily measured. A significant change may be positive proof of an effect on the reproductive tract and may occur within two or three months of the start of exposure. Conversely, semen specimens are difficult to obtain. The use of an appropriate reference group may be hard to ensure. A change in semen quality need not lead to abnormalities of reproduction. Past effects of exposure may not be detectable if exposure has been reduced or eliminated. Reproductive histories are readily obtained for fertility analysis. A reduction in fertility may be evidence of impaired health. Past effects of exposure are easily determined. For the detection of reduced fertility, however, two or three years of exposure may be needed. A change in fertility does not necessarily indicate an effect on the reproductive tract. The analysis of fertility requires considerable technical expertise. Since data for fertility analysis may be gathered in the workplace with little effort, suitable information should be obtained during employee medical examinations wherever potential exists for effects on reproduction.

Key terms: dibromochloropropane, indirect standardization, regression analysis, sperm count, sperm morphology, sperm motility, standardized fertility ratio.

Fertility, or the rate of producing children, depends upon the frequency of unprotected coitus, the ability to achieve fertilization, and the capacity for maintaining pregnancy to term. Increasing the quality of sperm motility and morphology will enhance fertility potential (10, 11). Sperm counts of less than $20 \cdot 10^6/\text{ml}$ are associated with reduced fertility, yet fertility is not affected by increases in count above this level (9). Of these parameters of semen quality, motility bears the strongest relationship to time needed for conception to occur. Sperm counts above $20 \cdot 10^6/\text{ml}$ and sperm morphology are apparently unrelated to ease of conception,

and, in cases of deficient sperm count but good motile activity, the time required to achieve conception is normal. While ejaculate volume tends to be greater among infertile men, it does not seem to be an important indicator of fertility or ease of conception (12).

Limitations to the use of semen in epidemiologic studies

A great deal of variability exists in the quantitation of semen quality. This situation reflects the inherent imprecision of the measurements, variation among observers, and differences between specimens obtained from a single donor at different times. For example, the 95 % confidence interval for sperm counts by three experienced technicians on a single semen specimen (mean count $29 \cdot 10^6/\text{ml}$) was ± 52 % (2). The power of a typical study to detect a mild or moderate effect on

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sperm count or motility is probably low. While sperm morphology appears to be less variable within individuals than motility or count (15), the application of classification criteria by traditional microscopic techniques has, of necessity, been subjective. Differences of 10 % between laboratories have been reported for average percentages of normal cells found among groups of fertile men (11). Negative results must always be accompanied by power estimates before conclusions can be generalized.

The analysis of semen, while helpful in determining reproductive function at the time of sampling, provides no information about past reproductive ability. Where reproductive dysfunction is accompanied by impotence or diminished libido, it would be difficult, if not impossible, to obtain samples. It is conceivable therefore that a situation might arise in which the persons most affected by occupational exposure would be unable to contribute specimens. Moreover, the testicular function of men who have had vasectomies cannot be ascertained from semen.

Since workers do not readily volunteer for semen studies, such epidemiologic investigations can be performed only after a great deal of concern has arisen. Even then, adequate participation cannot be assured. In situations where participation is substantially below 100 %, the possibility that the men available for study may not truly reflect the parent population is a matter for serious consideration.

A major problem is the identification of a suitable reference group. The participation rate of nonexposed persons desired as referents is usually much lower than that of exposed individuals. Volunteer referents are therefore less likely to be representative of potential controls than exposed participants are of exposed persons. Should the exposed group, for example, be found to have lower sperm counts, it may be difficult to determine whether it is they who have abnormally low counts or the reference volunteers whose sperm counts are unusually high and not typical of the entire potential reference population.

Distributions of sperm counts among normal men may be sought from the scientific literature without success. Those developed from aggregations of reference

groups are burdened by the issues already discussed. Others are available only for patients at fertility clinics, persons desiring vasectomies, or men whose wives have attended certain prenatal care clinics. The first group is less fertile than the general population, whereas the second and third are more fertile. Comparison with published values can be further complicated by differences in periods of abstinence preceding sample collection. The possibility that sperm counts are greater in winter than in summer raises new concerns (14). Published values may have been derived from specimens obtained at no particular time during the year and thus may be little affected by seasonal periodicity, whereas samples from individuals in a given study were most likely collected during one period.

Use of reproductive histories in epidemiologic studies

While semen quality is not synonymous with fertility, reproductive histories of workers' wives may be used to assess fertility directly. Participation is not a problem since over 95 % of the target population would be expected to take part in the less invasive questionnaire-based study. The necessary marital and reproductive information (dates of marriage and permanent separation, dates of birth of wives and their children) can be obtained from an individual in 3 min, and the worker may be interviewed instead of his wife. Work histories are derived from plant personnel records, which assure accuracy and minimize opportunity for confounding due to recall bias. A birth may be considered at risk from a particular exposure depending upon the worker's exposure status at the time of conception. Dates of conception are estimated by subtracting nine months from dates of birth.

The effect of exposure on fertility can be evaluated by means of indirect standardization or by regression analysis. With the use of indirect standardization, each person-year of marriage or fraction thereof is assigned an expected probability of birth taken from the US general population tables specific to race, age, parity, and birth cohort. Person-year contributions are determined separately for exposed and nonexposed periods, and expected num-

bers of births are computed for each woman specific to her husband's exposure status. Observed and expected births are summed over all women and used to compute standardized fertility ratios or ratios of observed to expected births. An estimate of the change in fertility concurrent with exposure (θ) is provided by the ratio of the exposed workers' to the non-exposed workers' standardized fertility ratios. Only experience at parities one or greater ($\text{parity} \geq 1$) is used for this estimate since the parity zero portion of tabled US birth probabilities underestimates the number of births expected for married women. The extent of parity zero experience and the degree of underestimation depends on age. The effect of exposure may therefore be confounded by age if the age distributions differ for the person-years of the exposed and nonexposed workers, as commonly occurs (5, 6, 13).

For application in regression analysis each person-year of marriage is identified with the wife's age, birth cohort, and parity as of January 1 and with the worker's race and exposure status. A year is designated as "exposed" if the employee has spent at least six months working in a part of the plant with potential for exposure to the agent of interest. The dependent variable is the occurrence or absence of a birth during a person-year. Race of the employee and maternal age, parity, and birth cohort are known to affect the probability of giving birth and are included in the model as independent variables. Ranges of values of the independent variables are ascertained separately for exposed and nonexposed experience. Person-years with any values outside the common ranges are excluded from analysis to make the distributions of experience more comparable. The result of the regression analysis is an estimate of the relative risk or odds ratio of giving birth in the presence or absence of exposure (1).

Certain limitations of fertility analyses must be pointed out. Fertility is influenced by social and psychological, as well as biological, factors. Thus a change in fertility does not necessarily indicate a biological effect. Frequent or chronic illness possibly related to exposure can reduce fertility although the reproductive tract may be unaffected. The reproductive experience of the wife is used as a surrogate for

the husband. While this procedure is not expected to cause confounding, it is not as reliable an indicator of the health of the worker as is a measurement taken exclusively from that individual. The reproductive capabilities of persons who have never married or of employees when their wives are past childbearing age cannot be ascertained. Moreover the analysis of fertility requires considerable technical expertise, and access to a computer and specialized software is highly desirable.

While contraceptive status can be accounted for directly in a regression analysis, its effects are more difficult to evaluate by means of indirect standardization since birth rates are not contraceptive specific. Explicit control for surgical sterilization, for example, has been shown to augment rather than diminish confounding error (7, 13). Whatever the contraceptive practices of the study group, confounding will occur only if the practices vary with exposure in a manner that is not controlled by indirect standardization. Additional assurance may be sought that confounding has neither produced a reduction in fertility with exposure nor obscured it. Exposure-related fertility may be compared not only with the preexposed experience of exposed persons, but also with the reproductive performance of a nonexposed reference group. Experience under study can be restricted to employed years in order to mitigate possible effects of unemployment. Patterns of contraception among the study group may be assessed in relation to general population data to determine if and to what extent relative usage might have changed with exposure.

If it were possible to minimize measurement variability, semen might be a more sensitive indicator of an occupational effect than reproductive histories, mainly because exposure sufficient to produce a mild decrease in sperm count would not reduce fertility should the concentration of spermatozoa remain above $20 \cdot 10^6/\text{ml}$. A significant change in semen quality may provide positive proof of an effect on the reproductive tract, while a change in fertility would not. Before fertility can be assessed from reproductive histories, at least nine months must elapse from the start of exposure, and two or three years may be needed to provide adequate power to detect a reduction (5, 7). Since effects on

fertility can only be described until nine months prior to the date reproductive histories are obtained, analysis is always retrospective. The analysis of semen, on the other hand, might be able to demonstrate a change occurring within two or three months of the start of exposure. [The time needed for spermatozoa to be produced from pale type A spermatogonial cells is 74 d (3).] Evidence of past reproductive impairment may not be detected, however, for semen studies tend to be cross-sectional or prospective in nature.

Selection of appropriate methods for epidemiologic studies

Analytical strengths and weaknesses should influence the selection of methods for evaluating occupational effects on male reproductive capacity. If, for example, fewer than two years have elapsed since the start of exposure, available data would probably be insufficient to detect an effect on fertility unless the group under study were very large. Semen, then, would be the preferred vehicle of analysis. Were the group too young to have accumulated much married experience or too old to have accrued sufficient exposed experience during childbearing years, a

semen study would again be indicated. On the other hand, because of the regenerative capacity of the germinal epithelium, if exposure has ceased altogether or diminished greatly for two or more years, fertility should be used as the primary information source. A rapid means of estimating the power of a proposed analysis of fertility has been described (6). Either method may be suitably employed in other instances, but, since reproductive histories are easy to obtain and one never knows in advance the extent of participation in semen studies, data suitable for analyzing fertility should always be collected. A positive epidemiologic study must be followed by thorough individual medical examinations.

A study at a chemical plant in Denver, Colorado, was one in which semen analysis was not the preferred method to demonstrate an occupational effect. Dibromochloropropane had been manufactured there since 1955. Production terminated in January 1976, yet semen samples and accompanying medical histories were obtained almost two years later. Except for a greater prevalence of azoospermia among the exposed, sperm count distributions of the exposed and reference groups were virtually identical (fig 1) (8).

An analysis of fertility was performed utilizing information collected from 60 exposed men during the course of a testicular function study (5). Data taken for analysis included employee birthdate, race, marital status, date and duration of last marriage, birthdates of spouse and children, previous marital status of spouse, and date the medical history was obtained. In addition the company provided the following information for each employee: plant date of hire; calendar years jobs were held; and, for each calendar year listed, hours worked with potential for dibromochloropropane exposure.

The fertility of the wives of male employees during marriage was analyzed according to the method of Levine et al (6), as modified by Starr & Levine (13). Exact dates of exposure were not available, but the number of hours exposed per calendar year was known for each individual. Because exposure could have taken place at any time during the year, a worker who received even a single hour of dibromochloropropane exposure was

Fig 1. Cumulative frequency distribution curve of sperm densities in exposed (broken line) and nonexposed (solid line) employees. [Reprinted from Lipschultz et al (8), copyright by Williams & Wilkins Company]

considered exposed for the entire calendar year.

Table 1 presents cumulative reproductive experience at parity ≥ 1 after employment at the plant, starting with the date the medical histories were obtained (1977) and retrogressing three years at a time until 1959. Cumulative reproductive experience to 1958 is also included. In the absence of exposure to dibromochloropropane, standardized fertility ratios consistently exceeded those under conditions of exposure. The differences were statistically significant as far back as 1959. Stable estimates of theta (only postemployment experience being used with at least five expected births in the denominators of each standardized fertility ratio) ranged from 0.11 to 0.50, values indicating a cumulative loss, in different years, of parity ≥ 1 fertility ranging from 50 to 89 % during exposure. In every instance the standardized fertility ratios for the nonexposed years following exposure exceeded those during exposure. Fertility returned to normal, therefore, after exposure ceased, although additional data suggest that it remained subnormal for about two years (5). These observations support the findings of Lantz et al (4) and others, who demonstrated that sperm counts of workers improved within two years following the last dibromochloropropane contact.

The strength of the fertility analysis, as applied to the experience of this workforce, was impressive. Data were obtained from reproductive histories which were designed for a testicular function study and often could not be used for the present purpose. As is typical of semen studies,

only about half of the exposed employees participated. Fertility analysis, therefore, was limited to data from one-third of the exposed workforce available in 1977. By way of contrast over 95 % of the target population can be expected to take part in the usual questionnaire-based fertility study. Moreover, if information had been collected at repeated intervals, the experience of persons who left the workforce would have been obtained.

In conclusion, salient features of the use of semen and reproductive histories are reviewed in the present communication. Traditional parameters of semen are easily measured. A significant change may be positive proof of an effect on the reproductive tract and may occur within two or three months of the start of exposure. Conversely, semen is difficult to obtain. The use of an appropriate reference group may be hard to ensure. The testicular function of men who are impotent or who have had vasectomies cannot be ascertained. A change in semen quality need not lead to abnormalities of reproduction. Past effects may not be detectable if exposure has been reduced or eliminated.

Reproductive histories are readily obtained for fertility analysis. A reduction in fertility may be evidence of impaired health. Past effects of exposure are easily determined. For the detection of reduced fertility, on the other hand, two or three years of exposure may be needed. A change in fertility does not necessarily indicate an effect on the reproductive tract. Fertility cannot be assessed for persons who have never married or whose wives are past childbearing age. The

Table 1. Cumulative fertility at parity ≥ 1 by calendar year.

Year	Postemployment									p-Value (one- tailed) X ₁ , X ₂
	X ₁ Nonexposed years preceding exposure			X ₂ Exposed years			Nonexposed years following exposure			
	Ob- served	Ex- pected	Standard- ized fertility ratio	Ob- served	Ex- pected	Standard- ized fertility ratio	Ob- served	Ex- pected	Standard- ized fertility ratio	
1977	30	22.5	1.33	11	17.5	0.63	8	6.8	1.18	0.02
1974	30	22.2	1.35	10	14.9	0.67	7	5.5	1.27	0.03
1971	30	21.8	1.38	7	12.0	0.58	6	4.8	1.25	0.02
1968	29	19.8	1.46	3	9.8	0.31	6	4.2	1.43	<0.01
1965	27	17.7	1.53	3	7.8	0.38	5	3.3	1.52	<0.01
1962	26	15.7	1.66	1	5.7	0.17	5	2.5	2.03	<0.01
1959	25	13.6	1.84	1	3.2	0.31	2	0.7	2.74	0.03
1958	24	12.8	1.88	1	2.0	0.50	1	0.3	3.61	0.13

analysis of fertility requires considerable technical expertise.

The concern of occupational physicians and, above all, of employees will determine the situations in which semen should be collected. However, since data for fertility analysis may be gathered in the workplace with little effort, suitable information should be obtained during periodic medical examinations wherever potential exists for effects on reproduction.²

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² Questions that may be incorporated into medical histories are available from the author on request.

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