Selection Bias in Occupational Sperm Studies

Solveig Brixen Larsen, Annette Abell, and Jens Peter Bonde

Participation rates in sperm studies are typically 25–50%, and therefore it is a matter of concern whether the men who provide semen samples truly reflect the parent population. The authors analyzed data from three Danish occupational sperm studies and evaluated the relation between age and occurrence of subfertility to participation and provision of semen samples. Age and subfertility were found to be rather strong determinants of participation. Willingness to provide semen samples was greater among men aged <40 years (odds ratio (OR) = 1.8, 95% confidence interval (CI) 1.3–2.6) and among men who had experienced an infertile period (OR = 1.7, 95% CI 1.3–2.1). Furthermore, the effect of infertility was modified by occupational exposure status, thus resulting in a tendency to differential selection and possibly biased risk estimates. In a cross-sectional study, the authors recommend that priority be given to a high participation rate and that data on the basic variables be collected from the entire study population, so that it is possible to make a nonresponder analysis and evaluate bias. Such an analysis can be carried out by means of questions enquiring about infertility, genital disorders, and earlier seminal examinations. Although the longitudinal study design is without many of the limitations of the cross-sectional study, a longitudinal study is usually not feasible. Am J Epidemiol 1998;147:681–5.
tionnaires and collect from 2 to 6 semen samples by masturbation after a recommended abstinence period (except the greenhouse worker study, in which no specified duration of sexual continence was requested).

Study groups

Farmers (unpublished study by Solveig Brixen Larsen, Aarhus University Hospital, 1996). One objective of this cross-sectional study was to find out if exposure to pesticides influences semen quality within a group of traditional and organic farmers. An invitation with 12 questions was mailed to 1,061 farmers (743 traditional and 318 organic farmers) selected from the Danish Ministry of Agriculture list of farmers. A total of 904 farmers (85 percent) returned the questionnaire; 102 were excluded because of vasectomy, and 327 (34 percent) agreed to provide two semen samples—one before and one after the spraying season. The participation rate was 28 percent among traditional farmers and 49 percent among organic farmers. The questions dealt with reproductive history and use of pesticides. The men were asked if “they had ever tried to obtain a pregnancy for at least 6 months without success.” It was possible to obtain the ages of almost all nonresponders from the register.

Metalworkers and electricians (8, 1987). One purpose of this study was to test the hypothesis that welding is detrimental to the male reproductive system. Six workplaces were selected, and a questionnaire was mailed to 672 male workers. The response rate for the questionnaire was 80 percent (n = 537). Forty-seven men were excluded because of vasectomy, and 229 (37 percent) agreed to deliver 3–6 semen samples. The participation rate was 38 percent in the exposed group and 35 percent in the control group. Information on age was obtained from the whole group (n = 625). The questionnaire provided data on reproductive experience and urogenital disorders (“have you ever tried to obtain a pregnancy for at least two years without success?,” “number of testicles in scrotum,” “treatment of cryptorchidism,” “orchitis as adult,” and “earlier genital operation”).

Greenhouse workers and members of an organic farmers’ association (unpublished study by Annette Abell, Aarhus University Hospital, 1994). The aim of this study was to determine if pesticide exposure in greenhouses entails a risk for reduced semen quality. A total of 211 greenhouse workers from 32 greenhouses were personally contacted and asked to participate. Only men aged 18–45 years were included. Twelve men were vasectomied, and 20 could not be contacted. The participation rate was 62 percent; 124 men agreed to provide four semen samples during one year of follow-up. Participants at a seminar held by the Danish Organic Farmers Association provided a control group. Among 43 seminar attenders, 32 agreed to participate (74 percent). All were asked about age and earlier genital disease (treatment of cryptorchidism, number of testicles in scrotum, and genital operations also including herniotomy). No data about fertility problems were collected.

Data analysis

First, we analyzed the three populations separately to find out if men who agreed to provide semen samples were different from nonparticipants with respect to age, occurrence of subfertility, and genital disorders. Odds ratios with 95 percent confidence intervals were calculated. The chi-square test for trend (9) was used to test whether the participation rates decreased as a function of age. Second, we examined whether a possible selection was related to occupational exposure status (exposed group vs. nonexposed group). The Breslow-Day test was used to test for homogeneity of the odds ratios across the strata (10). Finally, data from the farmer and metalworker studies were pooled (in the greenhouse worker study, there were no data on fertility), and logistic regression (11) was carried out to study the association between participation and the independent variables “age” (<40 years, ≥40 years) and “ever fertility problems” (no pregnancy within 6 months (farmer study) or 2 years (metalworker study) of unprotected intercourse). The model included an estimation of variation of selection across population study groups. The interaction between “age” and “ever fertility problems” was also evaluated in order to find out whether men with reduced fecundity were more interested in providing a semen sample when in “childbearing age” than when older. The SAS statistical package was used for the data analysis (SAS Institute, Cary, North Carolina).

RESULTS

Table 1 shows that the study participation rate decreased with increasing age in the farmer and metalworker studies. In the greenhouse worker study, participation rate was considerably higher than in the other two populations and was considered to be unrelated to age. Among farmers and metalworkers, participation rate increased from the youngest age group to 45 percent and 53 percent, respectively, but decreased to 30 percent and 18 percent, respectively, in the age group above 45 years.

Selection due to subfertility was found in both populations. Table 2 shows that men who had experienced an infertile period of at least 6 months (farmers) or 2
Selection Bias in Occupational Sperm Studies

TABLE 1. Participation rates in three Danish occupational sperm studies, by age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Farmers (n = 920)</th>
<th>Metalworkers (n = 624)</th>
<th>Greenhouse workers (n = 219)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>18-24</td>
<td>0</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>25-29</td>
<td>20</td>
<td>38</td>
<td>54</td>
</tr>
<tr>
<td>30-34</td>
<td>71</td>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>35-39</td>
<td>95</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>40-44</td>
<td>62</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>≥45</td>
<td>79</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>327</td>
<td>9.1</td>
<td>229</td>
</tr>
</tbody>
</table>

χ² (p < 0.01) 20.1 (p < 0.01) 0.02 (p = 0.88)

* Missing data: farmers (n = 39), metalworkers (n = 1), greenhouse workers (n = 27).

years (metalworkers) were more interested in providing semen samples. Table 2 also indicates differential selection according to exposure. Among farmers, this tendency was more pronounced in traditional than organic farmers (odds ratio (OR) = 1.9 vs. 0.95, p = 0.11). However, among welders, the selection was more pronounced in the nonexposed than in the exposed group (OR = 3.4 vs. 2.2, p = 0.48).

Data from the “farmer” and “metalworker” studies were pooled, and results from the logistic regression are shown in Table 3. Both age and subfertility were significantly associated with participation. Men with reduced fertility might be more interested in providing a semen sample if they were in childbearing age than if older. However, the relation between subfertility and willingness to provide a semen sample was not modified by age (OR for the interaction term = 1.2, 95 percent confidence interval 0.6-2.5).

A significantly larger percent of the participating greenhouse workers reported genital disorders compared with the nonparticipating men (17 vs. 6 percent, OR = 3.2, 95 percent CI 1.1-9.0). A similar tendency was present among the metalworkers (7 vs. 5 percent, OR = 1.6, 95 percent CI 0.7-3.4).

DISCUSSION

We found age and subfertility to be rather strong determinants of participation in the occupational sperm studies that we analyzed. Willingness to provide semen samples was higher among men aged less than 40 years and in the group of men who had experienced an infertile period. Furthermore, the effect of infertility was modified by occupational exposure status, thus resulting in a tendency to differential selection and possibly biased risk estimates. In the metalworker study, the selection on subfertility was most pronounced in the nonexposed group, leading to a bias toward the null hypothesis. Therefore, the reported relation to welding dust and semen quality was found in spite of this selection bias. However, this effect was only detectable in the high level exposed group of welders (12). Among farmers, the differential selection according to subfertility may introduce a bias against the null hypothesis.

Among traditional farmers, the proportion who provided semen samples was higher in men with indications of reduced fertility, but this trend was not found among organic farmers. The organic farmers may have an interest in reporting a high sperm quality and therefore less interest in participating if they suspected that the quality of their semen was poor. By contrast, subfertile traditional farmers might participate to a greater extent in order to obtain an evaluation of possible hazardous workplace exposures. Our results are in agreement with another sperm study (13) in which the prevalence of previous genital disorders in men who consumed organically produced food was lower than in men in a control group. Among metalworkers, a higher proportion of subfertile men wanted to participate, and this tendency is in agreement with results from an occupational study of spontaneous abortions among women who used video display terminals (14). In that study, it was found that women who had experienced a spontaneous abortion were more willing to participate than women who gave birth to a normal child. However, these findings are contrary to some studies. For example in the Framingham Study (15), a population-based cohort study of heart diseases, the healthiest subjects wanted to participate (15). The tendency toward a greater participation of the “sick” is perhaps a characteristic of occupational studies, because the “sick” persons want to find out if workplace exposure can explain their problems.

Differential selection related to exposure, as well as to semen quality, is of particular interest because of the potential to cause biased risk estimates. Only a few men had had an earlier semen examination, so it is only possible to evaluate the selection in relation to
TABLE 3. Association between the willingness to participate and the independent variables, age, occurrence of subfertility, and population, among farmers and metalworkers participating in Danish occupational sperm studies

<table>
<thead>
<tr>
<th></th>
<th>Adjusted odds ratio*</th>
<th>95% CI†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever infertility</td>
<td>1.8</td>
<td>1.3–2.6</td>
</tr>
<tr>
<td>Age (&lt;40 years)</td>
<td>1.7</td>
<td>1.3–2.1</td>
</tr>
<tr>
<td>Population (farmers vs. metalworkers)</td>
<td>0.9</td>
<td>0.7–1.2</td>
</tr>
</tbody>
</table>

* Odds ratios are adjusted for the other variables in the table.
† CI, confidence interval.

factors associated with semen quality (4). In our studies, only 10 percent of the farmers and 8.7 percent of the metalworkers had had an earlier semen examination. Men may suspect and have greater possibility of a poor semen quality if they have experienced an infertile period. A man who is considered to be “sub-fertile” by increased time to pregnancy may have “normal” semen quality and the woman may be the “subfertile” partner. However, we suppose that the “subfertile” women may be randomly distributed in relation to the analyzed variables. Among young men without a reproductive history, occurrence of genital disorders may cause doubt about reproductive capability.

In studies with a preferential participation of sub-fertile men, we would expect a higher average semen quality with increasing participation rate. A tendency toward lower participation rates among controls has been described previously (3), and if the exposure of interest reduces sperm quality, this effect will be more difficult to detect, because the control group will have an excess of subfertile men and thus appear to be more like the exposed group.

There was declining participation with age among farmers and metalworkers. The high overall participation rate among greenhouse workers may explain the absence of age influence. The greenhouse workers had been personally contacted at their workplaces, while the two other populations were contacted by mail. This may explain the difference in participation rates. Personal contact and meetings at the workplace is preferable but not always feasible. Furthermore, only greenhouse workers under 45 years were included. It may be possible to obtain higher participation in cross-sectional studies by limiting the age of the study population, but this could prevent identification of effects resulting from long-term cumulative exposure.

The different age distribution between participants and nonparticipants among farmers and metalworkers could introduce bias if the selection was differential in relation to exposure group, and sperm quality was...
related to age. However, some studies of age and sperm parameters do not indicate an effect of age on sperm counts, at least not before age 50 years (16, 17).

The cross-sectional study design has been used in almost all male reproductive studies to date, but it has several limitations, including selection bias because of differential participation, problems in defining a suitable reference group, and lack of information about the time dimension of the cause-effect relation. In the three studies discussed here, we used a longitudinal design, in which semen samples in principle are collected before, during, and possibly after exposure to the risk factor of interest. Causal inference is based on relative or absolute change of semen values over time within, rather than between, men. However, it can be difficult to establish a longitudinal occupational study because of low or very high turnover in the workforce.

During recent years, several papers (5, 18–20) have been published concerning semen quality in various populations and countries. The results of these studies indicate that great care must be taken when comparing results of cross-sectionally sampled semen studies from various sites. In order not to promote premature hypotheses and conclusions, it seems important to take into account the selection mechanisms and comparability of the different studies.

In conclusion, the results of our study suggest that both age and fertility have impact on participation in sperm studies. If differential selection takes place depending on factors related to semen quality, the internal validity may be affected. In a cross-sectional study, it is recommended that priority be given to a high participation rate and data collected on the basic variables from the entire study population, so that it is possible to make a nonresponder analysis and evaluate bias. The initial questionnaire should therefore include questions concerning infertility, genital disorders, and earlier seminal examinations. One could consider to collect blood samples from the whole population for inhibin B and follicle-stimulating hormone (FSH) analysis, because studies have suggested that serum inhibin B may serve as a direct marker of spermatogenesis in addition to FSH, which has its limitations as an indirect marker of testicular function (21, 22). The longitudinal study design is without many of the limitations of cross-sectional studies, but a longitudinal study is often less feasible to implement.

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REFERENCES