Are volunteers delivering semen samples in fertility studies a biased population?

F.Eustache¹, J.Auger¹, D.Cabrol² and P.Jouannet¹,³

¹Service de Biologie de la Reproduction–CECOS, Hôpital Cochin, Université Paris V, 75014 Paris and ²Service de Gynécologie–Obstétrique I, Hôpital Cochin, 75014, Paris, France

³To whom correspondence should be addressed at: Service de Biologie de la Reproduction–CECOS, Hôpital Cochin, Université Paris V, 75014 Paris, France. E-mail: pierre.jouannet@cch.ap-hop-paris.fr

BACKGROUND: It is not known to what extent the results of epidemiological studies on male fertility and semen quality based on voluntary participation in the general population are relevant. METHODS: In a study on the reproductive health of male partners of pregnant women, information was obtained from a group of men agreeing to collect a semen sample and to complete a questionnaire (group A), a group only completing the questionnaire (group B) and from men refusing to participate altogether (group C). RESULTS: The participation rate (group A) was 15.8% for 1409 men approached. Ages and socio-professional status were similar in the three groups. Time to pregnancy (TTP) was not significantly different in groups A and B, although there appeared to be an insignificantly higher proportion of couples taking longer than 12 months to conceive in group A than in group B. A history of urogenital disease appeared to be more frequent in groups A and B than in the general population. However, comparable semen characteristics were found for men with or without a history of urogenital disease. Pregnancy outcomes were similar in the three groups. CONCLUSIONS: The present study does not suggest major selection bias, although the social and reproductive histories of these men may prompt them to participate. Such factors need to be accounted for in similar studies.

Key words: environmental exposure/fertile men/recruitment bias/spermatozoa/TTP

Introduction

In the late 1990s, a cross-sectional study was undertaken to investigate geographical differences in semen quality in four European cities, Turku, Copenhagen, Edinburgh and Paris (Jørgensen et al., 2001). This approach was an improvement compared to retrospective studies because it was based on a precise protocol and the use of standardized procedures. However, several sources of bias may have been introduced, as the participation rates were 19% in Turku, 15% in Paris and 43% in Copenhagen. In Edinburgh, the inclusion process did not allow the participation rate to be calculated. Nevertheless, the geographical variations in semen quality found in this European study were too great to be accounted for by selection bias alone (Jørgensen et al., 2001).

The role of selection and confounding factors in epidemiological studies based on semen collection could be particularly important, as volunteers may tend to be those who are concerned about their fertility either because of previous urogenital, especially testicular, disorders or suspected infertility (Handelsman 1997; Larsen et al., 1998; Cohn et al., 2002). However, other sources of selection bias could arise such as financial compensation or not, socioprofessional status, length of questionnaires and medical history. No data have been published on the methodological or selection factors that may interfere in fertility studies of voluntary populations, especially when the study is based on self-administered questionnaires and requires semen collection.

During the European study (Jørgensen et al., 2001), detailed information on the male partners and on the pregnancies was collected for the Parisian couples who completed the questionnaire but did not give a semen sample and for those who refused to participate in the study. The aim of our study was to determine if the characteristics of the couples, men, and pregnancies, differed between the three groups of men studied, and to assess possible selection biases.

Methods

Study population

Pregnant women visiting the Obstetrics and Gynaecology department of Cochin Hospital (central Paris) for antenatal care from December 1996 to December 1997 received oral and written information about the study. After ensuring that the couples satisfied the inclusion criteria, they were invited to participate in the study. This required the collection of a semen sample. The eligibility criteria for inclusion were male partner aged 20–45 years at the time of invitation, born in France and couples living in the local referral area of the hospital. Furthermore, couples were excluded if the current pregnancy was the result of fertility treatment. Couples were not
excluded if they had a history of urogenital disease or if they had previously received fertility treatment (i.e. not for the current pregnancy). The questionnaires and protocol have been described in detail elsewhere (Jørgensen et al., 2001). Briefly, the questionnaires for both partners included information on age, occupation, medical history, lifestyle, starting time of the conception attempt for the current and any previous pregnancies (allowing the calculation of the time to pregnancy, TTP). The TTP questions were phrased as in the European Studies of Infertility and Subfecundity (Juul et al., 1999).

During the days following the first contact, the male partners were contacted by telephone and asked to attend the andrology laboratory to deliver semen and blood samples, and to have a physical examination. Men who accepted returned the questionnaires when they came to the laboratory (group A). Other men agreed to complete the questionnaire but did not agree to deliver a semen sample (group B). In this case, the couple sent the completed questionnaires by mail. A last group of men did not complete the questionnaire or give a semen sample (group C). All the men included in the study signed an informed consent form that had been approved by the local ethics committee. Information on the age and socio-professional characteristics of the men in group C, and on the pregnancy and delivery outcome for all couples was collected from the files of the Obstetrics and Gynaecology department.

The European study protocol stipulated that all men fully participating (questionnaire + semen collection and physical examination) could receive financial compensation for their travel expenses and/or lost working hours. To assess the influence of this compensation on the participation rate, indemnity (30 euros) was proposed to only one out of two approached participants in Paris.

Semen characteristics

A single semen sample was collected from each man by masturbation and ejaculation into a clean collection tube. It was recommended that semen samples should be collected after a minimum of 48 h but not more than 7 days of ejaculation abstinence (WHO, 1992). Semen volume, sperm concentration and motility were measured in one laboratory in each city, as described previously (Jørgensen et al., 2001). Sperm morphology was assessed in Paris using the method described by David et al. (1975) and modified after the report of Jouannet et al. (1988).

Endpoints and statistics

The data on the socio-professional category of the men approached are presented with the theoretical frequencies of each category in the general population living in the referral area of the hospital using the standardized categories of the French institute of statistics and economic studies (INSEE). We also studied if the age of both partners and some variables related to the pregnancy differed between the three groups. Unfortunately, it was not possible to obtain information on TTP for group C since in the routine pregnancy follow-up this information is not recorded in a standardized manner, or is not recorded at all. Moreover, no information about the medical history of the male partners was available for this group. One of our main aims was to determine if the men in group A had some specific reason for participating. Thus, we studied the answers to questions related to the men’s medical history and lifestyle by comparing the frequencies of positive answers to these questions in groups A and B. Pairwise differences were tested by the Mann–Whitney rank-sum test for the quantitative variables—except for TTP in groups A and B which were compared using the log-rank test—and by the Pearson Chi Square test for the qualitative variables. For these variables, lower and upper 95% confidence limits were calculated from the binomial distributions. When required, median and 25th and 75th values are presented. All statistical tests were carried out using the BMDP statistical software (Dixon, 1988).

Results

Participation

Figure 1 gives a flow chart of the number of couples who participated and did not participate in the study. Less than 0.5% of the couples refused to participate when they were first approached. The questionnaires were delivered to a total of 1409 couples. The study was mainly proposed to the woman, as the male partner was not present at the antenatal

![Figure 1](image-url)
care visit in ~95% of cases. Among the male partners of the 1409 couples contacted, 91 did not meet all the inclusion criteria, 208 men (15.8%) gave a semen sample and completed the questionnaire (group A), and 390 (29.6%) only sent back the completed questionnaire (group B).

Financial compensation was proposed to 699 couples approached (49.6%). Among these couples, 292 (41.7%) only sent back the questionnaires and 97 men (13.9%) also collected a semen sample. When the financial compensation was not proposed, a similar proportion of men collected a semen sample (101/710, 14.2%). Interestingly, 71 of the 97 men (73%) did not ask for the financial compensation proposed and those who accepted it belonged to various socio-professional categories and not only to those with the lowest purchasing powers (unemployed, student, blue collar workers etc.; data not shown).

**Socio-professional categories**

A higher proportion of the men who gave a semen sample had a high educational level (degree or still student: 78.9% for group A vs 69.2% for group B).

The distribution of men according to the standardized socio-professional categories is presented in Table I. In all three groups, almost half of the men were senior executives or had a ‘liberal’ profession (e.g. medical doctors, lawyers, self-employed). The socio-professional distribution in group A was not significantly different from that in groups B or C. For group A, the distributions were close to the published INSEE statistics for the four Paris districts near to the hospital, except for the proportions of senior executives that were marginally higher and unemployed men notably less represented.

### Parents’ history during pregnancy and medical history of the men

The answers to questions relating to the male partners’ mothers’ pregnancies, parent lifestyle during this period and disease history of the men during infancy are presented in Table II. Overall, no differences between groups A and B were found.

Similar numbers of men in groups A and B had a history of health problems. For example, diabetes mellitus was found in 0.5% vs 1.0% of men in groups A and B, respectively, and thyroid disease was found in 0% vs 0.5%, respectively.

### Urogenital history

Similar numbers of men in groups A and B had a history of urogenital disease like cryptorchidism, infection (gonorrhoea

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### Table I. Percentage [95% confidence limits] of fertile men participating or not in the study according to their socio-professional status and INSEE reference data

<table>
<thead>
<tr>
<th>INSEE Socio-professional categories</th>
<th>Study in Parisian male partners of pregnant women (prospective, 1996–97)</th>
<th>INSEE statistics (1990)(^c) for the four districts of Paris near to the hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A(^b) (n = 208)</td>
<td>Group B(^b) (n = 390)</td>
</tr>
<tr>
<td>Craftsman/tradesman</td>
<td>5.5 [3.1–8.9]</td>
<td>3.6 [2.2–5.8]</td>
</tr>
<tr>
<td>Senior executive</td>
<td>51.8 [45.7–57.7]</td>
<td>49.5 [45.1–54.1]</td>
</tr>
<tr>
<td>Officer/teacher/employee</td>
<td>35.3 [29.7–41.2]</td>
<td>38.8 [34.4–43.2]</td>
</tr>
<tr>
<td>Unemployed</td>
<td>3.5 [1.6–6.2]</td>
<td>9.9 [7.6–12.9]</td>
</tr>
</tbody>
</table>

Using the Chi-Square test, no significant difference between groups has been found.

\(^a\) Institut National de la Statistique et des Etudes Economiques.

\(^b\) A: participated and gave semen sample, B: participated but did not give semen sample, C: refused to participate.

\(^c\) 1990 data concerning the head of the household regardless of age and fertility status (http://www.recensement.insee.fr).

### Table II. Medical and lifestyle factors in the two populations of men who participated in the study

<table>
<thead>
<tr>
<th>Questions</th>
<th>Group A(^a) (n = 208)</th>
<th>Group B(^a) (n = 390)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of answers</td>
<td>Don’t know + not answered (% total)</td>
</tr>
<tr>
<td></td>
<td>[95% confidence limits]</td>
<td>[95% confidence limits]</td>
</tr>
<tr>
<td>Did your mother go to work when she was pregnant with you?</td>
<td>Yes: 53.6 [47.7–59.5]</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did your mother receive any treatment when she was pregnant with you?</td>
<td>Yes: 7.3 [4.4–11.4]</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (g, mean ± SD)</td>
<td>3343 ± 528</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did you suffer from any prolonged disease during the first year of your life?</td>
<td>Yes: 19.5 [14.8–24.9]</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did your parents smoke at home during your infancy (one or both)?</td>
<td>Yes: 57.3 [51.3–63.1]</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^a\) A: participated and gave semen sample, B: participated but did not give semen sample.
and chlamydia), varicocele and prostatitis: 8.75% vs 5%, 5.5% vs 4.2%, 2.4% vs 0.75% and 1.5% vs 0.29%, respectively.

In total, 27.4% of men in group A reported at least one urogenital disease compared to 17.4% in group B (not significant). Did these high proportions of men with a history of urogenital disease affect the TTP and semen characteristics?

**Semen characteristics, TTP and urogenital history**

Semen characteristics were not significantly different between the men having or not having a history of at least one urogenital disease. Only those having a history of urogenital disease had a lower, but not significantly different, total sperm count (Table III). Seven percent of men with no history of urogenital disease had < 20 million sperm/ml compared to 8.9% of those with a history of urogenital disease. The percentage of men with < 50% progressively motile sperm was 49.6% in the group of men with no history of urogenital disease and 50% in those with a history of urogenital disease. The percentage of men with < 30% morphologically normal sperm did not differ between the two groups (8.5% versus 12.5%, respectively). TTP distributions were not significantly different between groups, while a higher proportion of TTP > 12 months was found when the men had a urogenital history.

**Current pregnancy**

Table IV shows the ages of the male and female partners, the TTP distribution and pregnancy characteristics (mean value ± SD and/or percentage with 95% lower and upper limits, 95% CL) for the three subpopulations.

**Discussion**

It is currently considered that only prospective or cross-sectional controlled studies of volunteers can establish the baseline levels of semen quality and fertility status, as measured by TTP, in a defined population of men (Slama et al., 2004).
However, very few studies meeting these criteria have been carried out (Zinaman et al., 2000; Jørgensen et al., 2001; Swan et al., 2003). The questions concerning the participation rates and the representativeness of the volunteers, inherent in any epidemiologic study in self-selected volunteers, are probably even more important when the protocol requires the man to collect semen by masturbation. Thus, it is imperative to know the participation rate and to record the characteristics of participating and non-participating men to evaluate their influence on the results.

The participation rate in this study, involving a population of male partners of pregnant women living in the Paris area, was low: 15.8% of men gave a semen sample and completed the questionnaire (group A) and 29.6% only completed the questionnaire (group B). Our study was part of a coordinated cross-sectional study carried out in three other European cities: Edinburgh (Scotland), Copenhagen (Denmark) and Turku (Finland). The participation rate was similar in Paris and Turku (19%) and much higher (43%) in Copenhagen. The inclusion process used in Edinburgh did not allow calculation of the participation rate. It should be pointed out that in Copenhagen, ~80% of the semen samples were collected at the subjects’ homes and subsequently delivered to the laboratory, whereas in Turku and Paris >80% of the semen samples were collected at the clinic (Jørgensen et al., 2001). High participation rate was not confirmed in another study among military conscripts from Copenhagen where only 16% of them agreed to deliver a semen sample at the hospital (Andersen et al., 2000). Similar participation rates were obtained in a study on partners of pregnant women in four US cities, in which semen was systematically collected in a clinic (Swan et al., 2003). Similar participation rates (16–23%) were found in occupational studies (exposed vs non-exposed) requiring semen collection in our hospital (Multigner L et al., unpublished data). In other epidemiological studies of human semen quality, the participation rate was higher: 32.4% (Larsen et al., 1998), 53% (Cohn et al., 2002) and 54% (Hjollund et al., 2002), but in all these cases the semen sample was collected at home. These results suggest that the conditions in which the semen sample is collected strongly affect the participation rate among the general population. Obviously, it is easier for a man to deliver a semen sample at home than in an impersonal room in a laboratory.

This result illustrates the fundamental questions raised by semen studies in volunteers from the general population: does a low participation rate indicate a selection bias for the recruited population? If yes, what factors govern this selection? Finally, what are the consequences on the results of such studies, or are these results valid due to the scientific aims of the study?

Of course, studies only based on TTP where high participation rates (typically 60–80%) are usually observed do not suffer this disadvantage (Joffe 1997; Stewart et al., 2001; Slama et al., 2004) while not devoid of other sources of bias (Weinberg et al., 1994). However, TTP and semen quality do not provide the same information on the male side of reproductive function (Slama et al., 2004). In this respect, the design of the European study which combined a semen and a blood collection with the answer to numerous items related to the man’s medical history, lifestyle, occupation or to the current pregnancy in a long questionnaire cannot be compared to the vast majority of the previously reported occupational or fertility studies only based on semen quality or TTP alone.

Unfortunately, the European study did not include a questionnaire for the men refusing to participate, to find out some of the reasons for refusal (Jørgensen et al., 2001). Nevertheless, the data recorded at the Paris antenatal clinic allows one to know the age and socio-professional status of participating and non participating men and to obtain information on the pregnancy outcome. This enabled us to study putative selection biases as discussed below.

**Financial compensation**

According to our protocol, we could study the effect of financial compensation on the participation rate. The participation rates were similar among men offered financial compensation and the others; and most of the men to whom an indemnity was offered refused it. We might have expected the men with the lowest purchasing powers (unemployed, students etc.) to be over-represented among those accepting the financial compensation but this was not the case. However, the indemnity offered was modest (30 euros, ~US$30). In a Danish study among military recruits (Andersen et al., 2000), the men received a more substantial financial reward (US$60), which did not seem to increase the participation rate (16% vs 15% in our study). In Paris, a large proportion of the men who agreed to participate claimed that it was the subject of the study, related to the influence of lifestyle and environmental factors on reproductive health, that had encouraged them to participate. All these findings suggest that the offer of financial compensation is not a strong motivation for the volunteers participating in semen studies, at least in France.

**Socio-professional status**

Overall, nearly half of the men, whatever the group, were senior executives or professionals. This proportion was markedly higher than that found in the French male population in the same age range (14.4%; http://www.recensement.insee.fr) but it was similar to that recorded in the administrative district of the hospital. However, when the men accepted to participate, the proportion of senior executives was higher than in the corresponding Parisian districts (see Table I). The other socio-economical categories did not differ between groups (except for the unemployed men) and were comparable to that recorded in the hospital area. Moreover, the respective proportions of men in each of the standardized socio-professional categories were close to those found in a retrospective study of healthy fertile men from the Paris area who donated their semen in an artificial insemination program (Auger et al., 1995). Our study involved a long questionnaire (20 pages) and the collection of a semen sample, thus, it is difficult to know whether socio-professional status was a major factor influencing the participation rate. According to Auger et al. (2001) and Jensen et al. (2001), socio-professional status can modulate semen characteristics.
and fertility. It is obvious that in a multicentric study in different geographical areas, this factor should be regarded as a major covariable. Further studies are warranted to assess the influence of socio-professional status on semen characteristics and fertility.

History of urogenital disease

Our findings suggest that men with a history of urogenital disease are more likely to participate in such studies, even if they are fertile. Moreover, the proportion of men with urogenital history was higher in the group of men who delivered a semen sample than in those who only completed the questionnaire. Overall, the frequencies of cryptorchidism and infection (gonorrhoea and chlamydia) appeared to be higher in group A than in the male population of developed countries: 8.75% versus 1–2% (Toppari and Kaleva, 1999) and 5.5% versus ~2% (Meyer et al., 1994; Pierpoint et al., 2000), respectively. On the contrary, the frequencies of varicocele or prostatitis were lower in group A (2.4% and 1.5%) than those reported for the general population, 8.2% (Gioffre et al., 2002) and 5–9% (Roberts and Jacobsen, 2000), respectively. A high frequency of urogenital history among participating men was also observed in the three other cities of the European study (Jørgensen et al., 2001).

Selection bias

We did not find any difference in the ages of the men or their partner, or in the TTP between the men who gave a semen sample and those who only answered the questionnaire. However, it should be pointed out that a higher proportion of long TTPs was found in the group of men who accepted collection of their semen. This result, together with the finding of a higher proportion of men having a history of urogenital disease, suggests that personal factors related to male reproductive health may prompt the men to participate. There were no statistical differences in the data related to the current pregnancy in the three groups of men. However, the findings of a marginally increased rate of pathological pregnancies in the groups B and C suggested that in this situation the male partners could be less inclined to participate.

In a recent US study of fertile men (Swan et al., 2003), 40% of the men who refused to participate answered a very brief mini-questionnaire that included items about demographic data, history of infertility and TTP, and a sample of study participants answered the same questions to compare responses and to examine selection bias. The authors did not find any significant difference between these groups. They also compared responses about fertility (whether either partner had ever seen a doctor for infertility) and TTP in a sample of volunteers and in subjects who refused to participate and found no differences (Swan et al., 2003). These findings argue against a noticeable selection bias.

We compared the semen characteristics and the TTP for the men with and without a history of urogenital disease and found no significant differences. However, given the small sample size, it is not possible to draw a general conclusion as some of these diseases, for example a history of undescended testicle, may affect semen quality (Mieuuset et al., 1995; Gracia et al., 2000; Auger et al., 2001) and possibly increase TTP (Jensen et al., 2001). Therefore, we can speculate that higher proportions of men with a history of urogenital disease might decrease the mean values of the semen characteristics and increase the TTP observed in the entire group of study subjects. Since the men who had a history of at least one urogenital disease had a smaller number of spermatozoa than those with no such history, it could be recommended that this variable be taken into account for adjustment in multicentre studies.

Conclusions

Our results indicate that socio-professional status and the offer of financial compensation do not have a strong effect on the participation rate in fertility and semen characteristics studies. Conversely, we found that a higher proportion of fertile volunteers with a history of urogenital disease participate in such studies, but this does not seem to have major consequences on semen characteristics. The men who gave a semen sample appear to be representative of the population in the referral area, reinforcing the biological and general relevance of data. In conclusion, despite the low participation rate, the present study does not indicate major selection bias. However, it suggests a marginal influence of social factors and factors related to the reproductive health of the men which prompt them to participate. Therefore, such factors need to be accounted for in fertility studies requiring semen samples from fertile male volunteers.

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References


