Factors associated with male infertility: a case–control study of 218 infertile and 240 fertile men

*Sin-Eng Chia Associate Professor, **Soon-Tiong Alvin Lim Senior Scientific Officer, **Sun-Kuee Tay Senior Consultant, **Swee-Tee Lim Senior Staff Nurse

*Department of Community, Occupational and Family Medicine, National University of Singapore; **Department of Obstetrics and Gynaecology, Singapore General Hospital

Objective To determine the likely risk factors, such as smoking and drinking habits, and occupational groups, for infertility in a group of infertile men with no known cause, compared with a group of fertile men; and to examine the effects of the semen parameters, such as volume, density, motility, viability and normal morphology, on fertility.

Design A case–control study.

Setting The department of obstetric and gynaecology of a tertiary general hospital.

Participants Six hundred and forty consecutive male partners of couples trying to conceive were recruited from an infertility clinic. Of these, the cases comprised 218 men who had no known cause for their infertility. Two hundred and forty men whose wives were pregnant at the time of the study were recruited as controls.

Results The semen parameters (e.g. density, total sperm counts, motility, viability and normal morphology) of all cases were significantly poorer than that of the controls. The risk of infertility is associated with smoking (crude OR 2.82, 95% CI 1.93–4.13; adjusted OR 2.96; 95% CI 1.98–4.42). Technicians (adjusted OR 2.81; 95% CI 1.51–4.24) and professional, senior officials and managers were also at a greater risk of infertility (adjusted OR 2.36; 95% CI 1.26–4.40), compared with service and clerical workers. The significant factors predicting infertility were smoking, density of sperm, and viability of sperm. Smoking increased the odds of being infertile. Higher sperm counts and larger percentage of viable sperm decrease the odds of infertility. Based on the multiple logistic regression model, the odds ratio for infertility = 94.70 × 2.88 smoking × 0.29 density × 0.95 viability.

Conclusion Smoking, density of sperm and the viability of sperm are significant predictors for infertility among men.

INTRODUCTION

Researchers estimated that 15% of couples in the United States alone are involuntarily infertile. In one-third of cases, infertility in the male is considered to be the predominant reason for a woman’s failure to conceive. Most subfertile men do not have a definable cause for their problem and only in approximately one-fifth can a clear cause be determined. Despite the significant contribution of the male factor to the infertile state, relatively little has been done to increase our knowledge in this field. In the 1970s and 1980s, the issue of male infertility was overshadowed by major advances in assisted fertilisation, such as in vitro fertilisation (IVF), or gamete transfer, techniques largely developed to circumvent the female factor infertility. It was only in the 1990s, with the reports of decline in sperm quality over the last few decades, that there has been a resurgence of interest in factors that may affect male infertility.

Most of the reported studies have used the case–control design in examining the risk factors associated with male infertility. This method is probably the better alternative short of the expensive and time consuming prospective cohort study. However, one shortcoming in most of these case–control studies is an appropriate control group. Most of these studies have drawn their controls from couples attending the infertility clinics. The selection criteria for fertility is based on the men’s semen parameters. The quality limit of semen parameters designed to discriminate infertile from fertile men is constantly being re-examined and redefined. Indeed, reports are often appearing in the literature of pregnancies occurring with men well below the subfertile...
threshold. In a five-year follow up of 1089 infertile couples, there was no significant difference in any of the semen variables between couples who remained infertile and those who later conceived.

Some other studies have recruited either sperm bank donors or volunteers who had fathered a child within the past year or more as their control (fertile) subjects. These criteria for fertility are also questionable. Sperm bank donors are highly selected populations. A survey was commissioned by the Human Fertilisation and Embryology Authority (HFEA) to determine the attitudes and motivations of semen donors compared with a matched group of nondonors. Cook and Golombok reported that despite the recommendation by the HFEA that attempts should be made to recruit semen donors from older men in stable relationships who already have children of their own and who wish to donate for altruistic reasons, it remains the case that the large majority of men in the UK who donate semen are young single students who are largely motivated by payment. Schover et al. likewise reported that most of their semen donors were motivated by financial compensation. Also, the semen result of any man who had fathered a child within the past year is at least 'one-year' old, and as such, it may not be good recent indicator of fertility.

The present study was based on a sufficiently large group of men (n = 240) of proven fertility (wives who are pregnant at the time of providing the semen). The findings are more likely to be an accurate reflection of sperm parameters and fertility. The aims of the study were: 1. to determine the likely risk factors, including smoking and drinking habits, and occupational groups, for infertility in a group of infertile men (with no known cause) compared with a group of fertile men; and 2. to examine the effects of the semen parameters, including volume, density, motility, viability and normal morphology, on fertility.

METHODS

Our case-control study was approved by the relevant hospital's ethical committee. Cases refer to the male partner who had been investigated for inability to conceive, for which there was no known medical conditions that could account for the infertility. The control refers to a fertile male, defined as someone whose wife at the time of the study was pregnant but had not delivered.

All male partners of couples who were undergoing initial screening for infertility in an obstetric and gynaecology department of a general hospital in Singapore were included in the study, and each person in the study was interviewed by a trained interviewer. A questionnaire was used to elicit:

1. Occupational exposure to agents that are known to affect spermatogenesis.
2. Alcoholic consumption.
3. History of smoking.
4. Past medical history.

History of smoking was classified into: nonsmoker (individuals who never smoked a cigarette before); ex-smoker (individuals who had quit for more than a year); smoker (anyone who was currently smoking). Smokers were also asked how many years they had smoked and the average numbers of cigarettes smoked per day. None gave a history of taking any hallucinatory drugs. Each subject's last occupation was classified according to the standard classification code of the Singapore Standard Occupational Classification. This was followed by a clinical examination by a gynaecologist.

Of a total of 640 male partners seen, 218 were included in the study having fulfilled the following criteria:

1. Absence of factors which could possibly influence male infertility (defined by the World Health Organisation Task Force on the Diagnosis and Treatment of Infertility) included a history of diabetes mellitus, long term medication, urinary tract infection, sexually transmitted diseases, and testicular injury.
2. No clinically detected abnormality; none of the men had small testes; none had either testicular volume < 10 mL (based on the clinical experience of andrologists) or varicoceles.
3. More than one year of failed attempts at conception.
4. Exclusion of female factors (e.g. tubal occlusion, endometriosis, pelvic inflammatory diseases, endocrine and ovulation defects).

Each subject was asked to cough when a Doppler stethoscope was placed against each side of the scrotum. A positive recording from the Doppler stethoscope would be indicative of the presence of varicoceles. Each of the 218 infertile men will henceforth be referred to as a case.

The controls were recruited after the cases were obtained. Women attending the antenatal clinic, at the same department, were approached by a female obstetric nurse. The purpose of the study was explained to the pregnant woman by the nurse, and if the woman agreed to the study, she would then seek her husband's consent to participate. Only couples who had never attended an assisted reproductive programme were recruited for the study. This information was verified with both the husband and wife, separately.

In all, 320 women were approached for the study. A total of 240 fertile men consented to the study giving a response rate of 75%. The most common reasons given by those who did not participate were: 'husband too busy', 'husband not interested', and 'I don't think my husband will be interested'. For these nonresponders, we were unable to determine the demographic features.
of the male partners. Those who consented to the study will henceforth be referred to as controls. All who agreed to participate in the study had to sign a consent form. Approval for the study has earlier been obtained through the relevant Hospital Ethics Committee. These fertile men were similarly interviewed by a trained interviewer using the same questionnaire that was used for the cases.

Semen collection and analysis

The men were asked to collect their semen at home in the morning by masturbation into a sterile wide-mouth plastic container, after three days of abstinence. The samples were brought into the hospital within one hour of collection. Time of ejaculation, abstinence period, spillage (if any), and fever during the last three months were recorded by the subject. All semen samples were processed and analysed by one experienced laboratory assistant at the Fertility Clinic of the Singapore General Hospital within one hour of receiving the samples. Volume, total sperm count, sperm viability, proportion of progressively motile sperm and proportion of normal and abnormal sperm forms were examined according to the WHO guidelines for the examination of human semen. Intra-specimen assays for all the above parameters consistently gave values within a 10% variance.

At least 100 sperm were assessed for each parameter. In routine motility assessments, sperm from a minimum of five fields were enumerated and classified into good progressive motility, sluggish motility and nonprogressive motility. Sperm morphology was determined after Papanicolaou staining of air-dried smears. The criteria applied in determining sperm morphology were as spelled out in WHO guidelines for the examination of human semen. Intra-specimen assays for all the above parameters consistently gave values within a 10% variance.

Statistical analysis

Log transformation was used (where necessary) for some of the semen parameters to improve the distribution of the data. Statistical analyses were performed with standard contingency tables and test of statistical significance (Fisher’s test and Student’s t test). Crude odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for case–control associations with factors suspected to affect fertility. The adjusted odds ratios were calculated using multiple logistic regression, adjusting for age, smoking habits, drinking habits and occupational groupings, where appropriate. To determine the significant contributions of the different factors towards infertility, another logistic regression model was determined based on values of a set of predictor independent variables. The variables (smoking habit, age, logarithm of density, logarithm of normal morphology, logarithm of volume, motility and viability) were entered in a stepwise forward conditional method. Only variables that contributed significantly to the final equation were accepted. Occupational group was not included in the model as it did not contribute significantly.

Statistical analysis was carried out using the SPSS 7.5 on a personal computer.

RESULTS

Table 1 shows the basic characteristics of the cases and controls. The men were similar in the mean age groups. The racial groups were also similar among the ‘Malays’ and ‘Others’. However, there are differences in the cases and controls for the Chinese and Indians. These differences are unlikely to affect the results as racial groupings have not been shown to be associated with abnormal semen parameters. The percentage of smokers was significantly higher among the cases compared with the controls (P < 0.000). Although the percentage of drinkers among the cases was greater than controls this difference was not significant. More than 70% of the wives of controls were in their first and second trimesters of pregnancy (range 2–39 gestational weeks) with a median and mode of 16 and 8 gestational weeks, respectively. None of the subjects was on drugs or other medications at the time of the study.

Despite the fact that the cases had no known causes for their infertility all their semen parameters, except for mean semen volumes, were significantly worse when compared with those of the controls (Table 2). The cases’ mean density, total sperm counts, motility, viabilit-

Table 1. Basic characteristics of the study population. Values are given as n, n (%) or mean [SD].

<table>
<thead>
<tr>
<th>FACTORS ASSOCIATED WITH MALE INFERTILITY</th>
<th>57</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of men</strong></td>
<td>Infertile men</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>34.4 [4.6]</td>
</tr>
<tr>
<td><strong>Alcohol intake</strong></td>
<td>Social drinkers*</td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Ethnic groups</strong></td>
<td>Chinese</td>
</tr>
<tr>
<td><strong>Week of pregnancy</strong></td>
<td>—</td>
</tr>
</tbody>
</table>

*Those who drink less than once a month, each time no more than two large bottles of beer.
Table 2. Semen parameters of the fertile and infertile men. Values are given as mean (range).

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>Infertile men (n = 218)</th>
<th>Fertile men (n = 240)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>2.6 (0.5-8.6)*</td>
<td>2.0 (0.2-8.6)*</td>
<td>0.000</td>
</tr>
<tr>
<td>Density (10^6 per mL)</td>
<td>14.8 (0.1-227)*</td>
<td>44.4 (1.6-433.0)*</td>
<td>0.000</td>
</tr>
<tr>
<td>Total sperm count (10^6)</td>
<td>39.0 (0.05-1952)*</td>
<td>88.8 (3.2-3724)*</td>
<td>0.000</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>45.4 (0-78)</td>
<td>54.9 (5-90)</td>
<td>0.000</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>58.9 (0-88)</td>
<td>73.6 (24-98)</td>
<td>0.000</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>13.9 (0-45)*</td>
<td>16.6 (2-53)*</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Geometric means.

ity and normal morphology were all below the normal values based on the WHO criteria of minimal normal semen parameters. Whereas, for the controls, all the mean semen parameters (except percentage of normal spermatozoa morphology) were above the WHO values of minimum normal semen parameters.

Table 3 shows the factors associated with fertility. Risk for infertility is associated with smoking (crude OR 2.82; 95% CI 1.93-4.13). This risk is still present even after adjusting for drinking status, age and occupational groups (adjusted OR 2.96; 95% CI 1.98-4.42). The smoking habits were divided into different groups by the numbers of cigarettes smoked multiplied by number of years of smoking. There were no significant differences among the different smoking groups. In the occupational groups, 'service and clerical workers' was taken as the reference as this group was least likely to be exposed to chemicals in the workplace that may affect spermatogenesis. With regard to psychological stresses, this group was probably the least likely to be stressed. Interestingly, the production workers were not at higher risk of infertility compared with the service and clerical workers. Technicians, however, has a significantly greater risk of infertility, adjusted OR 2.81 (95% CI 1.51-4.24). Professionals, senior officials and managers were also at a greater risk of infertility, adjusted OR 2.36 (95% CI 1.26-4.40).

All the significant risk factors (Table 3) in addition to the semen parameters were entered into the multiple logistic regression model in a stepwise forward conditional method. The significant factors that predicted infertility were smoking, density of sperm, and sperm viability (Table 4). Smoking increased the odds of being infertile. Higher sperm counts and a larger percentage of viable sperm decrease the odds of infertility. Table 5 shows the odds of infertility in men with different contributing factors; with smoking as the factor which greatly increases the risk of infertility.

**DISCUSSION**

To our knowledge, this is the first case–control study of

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*)Numbers do not add up to the total for cases and controls in the occupational groups, as some of the subjects did not give a complete answer to this question.

1)Adjusted for age, smoking and drinking status and occupational groups, by multiple logistic regression.

2)Adjusted for age, smoking and drinking status, by multiple logistic regression.

3)Significant odds ratios are those with lower 95% confidence values, greater than one.
fertility including a large group of men, with proven fertility and available semen parameters, as controls. The present study was based on a sufficiently large group of men (n = 240) of proven fertility (i.e. their wives were pregnant at the time of the study) providing semen. The findings are more likely to be an accurate reflection of sperm parameters and fertility. Ideally, it would have been better to obtain the men’s semen as soon as their wives become pregnant, but this would be very difficult to do logistically. Thus, it would be true to say that the sperm parameter measured in the present study may not be an absolute reflection of the subjects’ sperm quality at the time of conception. But the time lag is not too long considering that the median and mode were 16 and 8 gestation weeks, respectively.

There are other limitations inherent in this study. As the interviews were not performed blindly, a related bias cannot be ruled out. The cases may try to hide the fact that they were smokers and/or drinkers. If this were true, the estimated odds ratios for infertility would be lower than expected. Most of the other information obtained from the questionnaires is not likely to suffer from recall bias. Concerns about recall bias are more likely to be overrated in case-control studies of birth defects, spontaneous abortion or other fatal diseases. We were not able to determine the demographic characteristics of nonresponders. As such, the nonresponders may bias the results: for example, if the nonresponders knew that they had semen parameters that were poorer than those of the responders and therefore did not want to volunteer. This argument does not hold as the subjects would not have known their semen parameters prior to being approached. On the other hand, it may be that those who lead a healthier lifestyle (i.e. do not smoke and/or drink) are more likely to volunteer than those who do not. However, the subjects were not told which factors were being studied. Therefore, we do not think that possible self-selection biased the results significantly. A response rate of 75% could be considered as fairly representative of the sampled population.

In some countries only a minor fraction of infertile couples seek medical assistance, but this is not true in Singapore. Health care is affordable and assessable. Because of the norm of a two-child family, most couples would seek medical assistance if they had a problem trying to conceive.

Unmatched selection of controls was performed. Ideally, the subjects should have been matched for age and other factors that are known to affect fertility. But it is very difficult, throughout the world, to persuade healthy men to provide their sperm for analysis. If we were very selective in matching the controls, it would be very difficult to get the adequate sample size to obtain the necessary power for the study. The recruitment of the controls was strictly a random process. There was a homogeneous mix of patients (i.e. different socioeconomic status and occupations) who attended the obstetric clinic. The response rate of 75% is a good representation of the fertile men. None of the cases had a past or present history of any known medical conditions that could affect male fertility. Thus, known causes of male infertility can be ruled out among the cases. The controls, however, were not matched with regard to the medical history. There were about 20% of controls with significant past medical history, including sexually transmitted diseases, mumps and varicocele. However, there were no exceptional differences in any of the semen parameters between fertile men with a significant past medical history and those without a past medical history (data not shown).

Sperm counts are subject to wide variation, hence some researchers have questioned the use of a single sample for semen analysis. However, the report of Whorton and Meyer on sperm analysis in a large cohort of American agri-chemical workers, suggested that a single sample will suffice for an occupational study. Frequency of ejaculation is also a factor that affects the result of semen analysis and consequently, risk evaluation. This would not apply in our study. All the subjects had a three-day abstinence period, confirmed by the staff when the semen samples were collected.

It is clear that the cases had poorer semen parameters compared with the controls. Using the WHO criteria, the cases’ geometric mean semen parameter values were

\[
\frac{\text{In (odds ratio for infertility)}}{1.2380} = 4.5507 + (1.0591 \times \text{smoking}) - (1.2380 \times \text{logdensity}) - (0.0501 \times \text{viability})
\]

Odds ratio for infertility = 94-70 \times 1.2380 \times 0.2910 \times 0.95

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient (B)</th>
<th>Regression coefficients (R)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>1.0591</td>
<td>0.1818</td>
<td>2.88 (1.87-4.45)</td>
</tr>
<tr>
<td>Logdensity</td>
<td>1.2380</td>
<td>-0.2039</td>
<td>0.29 (0.18-0.46)</td>
</tr>
<tr>
<td>Viability</td>
<td>-0.0501</td>
<td>-0.2345</td>
<td>0.95 (0.94-0.97)</td>
</tr>
<tr>
<td>Constant</td>
<td>4.5507</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Regression equation:
\[
\ln (\text{odds ratio for infertility}) = 4.5507 + (1.0591 \times \text{smoking}) - (1.2380 \times \text{logdensity}) - (0.0501 \times \text{viability})
\]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking (0 = nonsmoker, 1 = smoker)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Density (millions/mL)</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>75</td>
<td>75</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.16</td>
<td>0.40</td>
<td>6.09</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Table 4. Significant factors that predict infertility.

Table 5. Odds of infertility for men with different factors based on the regression equation for infertility.
also below the normal values (Table 2). Seminal analysis results provided the basis to conclude that the cases were indeed infertile. Smoking was found to be a significant risk factor for infertility with a crude OR and adjusted OR of 2.82 and 2.96, respectively. The effect of smoking on sperm quality has been a subject of much discussion in the literature over the last decade. However, the findings have largely been contradictory. The reasons for these discrepancies are still unclear. It could be due to environmental and socioeconomic factors of the different countries where the studies were conducted. Different studies methodology could also be involved. Despite the variation in results of clinical studies, cumulative evidence is sufficient to implicate cigarette smoking as a causal factor in male subfertility. In a recent review on smoking and male reproduction, Vine concluded that although smokers as a group may not experience reduced fertility, men with marginal semen quality who wish to have children may benefit from quitting smoking. This conclusion is supported by our findings. Smoking is an important factor in increasing the risk for infertility. In fact, its contributions to infertility is far greater than the protective nature of increase sperm density and viability (Table 4). Although the details of the smoking habits were available there were no significant differences in cigarette consumption habits among the different groups.

Alcohol has been postulated to be a factor in male infertility, considering the frequent changes in testicular function associated with heavy drinking. Most recent studies suggest that alcohol consumption of < 40 g per day is unlikely to play a pivotal role in the aetiology of poor semen quality. Similarly, the present study showed that there were no significant differences in any of the semen parameters between the drinkers and nondrinkers. The drinkers in this study, both cases and controls, were social drinkers (i.e. those who drank less than once a month, each time no more than two large bottles of beer). As such, their exposure to alcohol was minimal. Alcoholic drinks are expensive in Singapore, and this high cost is probably the main reason for the low alcohol consumption among subjects in our study.

Occupation was not significantly associated with infertility, but when the different occupational groups were compared with the reference group (service and clerical workers) technicians and professionals were at greater risk of infertility (Table 3). When these two groups were combined and compared with the reference group, the adjusted OR was 2.58 (95% CI 1.46-4.56). It was thought that production workers would be the group most likely to be exposed to physical and/or chemical hazards at the workplace. However, they did not have a significant increased risk for infertility, compared with the reference group. The production workers from our study may not have been exposed to reproductive hazards at the workplace. Technicians and professionals were less likely to be exposed to agents that affect spermatogenesis (e.g. heat, lead, mercury, ionisation radiation). One would have thought that their risk for infertility would be less, but these groups may be more prone to psychological stress by virtue of their job responsibilities. Psychological stress has been demonstrated to cause depressed testosterone levels in humans and rodents, but the relationship between this effect and infertility is not clear. It has been suspected, and in some cases demonstrated, that stress can adversely affect reproduction function. Both animal and human data support this contention. However, the human data are clear in extreme situations (e.g. inmates of concentration camps) but less so under less drastic conditions. Fenster et al. investigated the relationship between psychological stress and human semen quality, and reported that stress at work and total number of life events were not related to differences in semen quality. As psychological stress was not measured in these subjects, any further inference may be too presumptive.

Seminal analysis is the test most widely used to estimate fertility potential in men. A male factor is considered when no abnormalities have been found in the woman and when sperm quality is below a particular standard. However, the quality limit designed to discriminate infertile from fertile men is constantly being re-examined and redefined. Thus earlier reports had suggested sperm concentration as a measure of fertility, while the later reports had proposed sperm morphology and the proportion of sperm with progressive movement as the decisive variables. The WHO had recommended that a normal semen sample should have '30% normal spermatozoa'. However, studies of fertile men have reported that more than half of their men had < 30% normal spermatozoa, a third of which was suggested sperm morphology as the decisive variables. The WHO criteria has not been identified uniformly as a predictive variable for success using in vitro fertilisation. Some have reported a positive relation to IVF outcome, whereas others showed no association at all. In in vivo conception, Barratt et al. concluded that 'morphology scoring at the light microscope level has only a weak association with in vivo conception'. Large percentages of normal spermatozoa may not be a significant factor for fertility. In our study we have found that the significant predictors of fertility were sperm density and viability among all the other semen parameters. To our knowledge, there are no published reports which present the odds of infertility given some known factors that may affect fertility. The equation in Table 4 may serve as a guide for doctors advising men who seek advice on
fertility. However, more studies would need to be conducted to further validate the results of this study.

Acknowledgement

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