Human Reproduction, Vol.27, No.9 pp. 2799–2806, 2012
Advanced Access publication on June 12, 2012 doi:10.1093/humrep/des183

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Modifiable and non-modifiable risk factors for poor semen quality: a case-referent study

A.C. Povey[1,*], J.-A. Clyma[1], R. McNamee[2], H.D. Moore[3], H. Baillie[3], A.A. Pacey[3], N.M. Cherry[4], and Participating Centres of Chaps-uk[†]

[1]Centre for Occupational and Environmental Health, School of Community-Based Medicine, Faculty of Medical and Human Sciences, University of Manchester, Manchester M13 9PL, UK [2]Health Methodology Research Group, University of Manchester, Manchester, UK [3]Academic Unit of Reproductive and Developmental Medicine, University of Sheffield, Sheffield, UK [4]Division of Occupational Medicine, University of Alberta, Alberta, Canada

*Correspondence address. Tel: +44-161-275-5232; Fax: +44-161-275-5595; E-mail: apovey@manchester.ac.uk

Submitted on January 6, 2012; resubmitted on March 23, 2012; accepted on April 26, 2012

STUDY QUESTION: Are common lifestyle factors associated with low-motile sperm concentration (MSC)?

SUMMARY ANSWER: Common lifestyle choices make little contribution to the risk of low MSC.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: Reviews of male subfertility often highlight how aspects of men's adult lifestyle can significantly increase their risk of subfertility but the strength of supporting evidence is weak. In this study, although low MSC was associated with a history of testicular surgery, being in manual work, not wearing loose underwear and black ethnicity, no relation was found to consumption of alcohol, use of tobacco or recreational drugs or high body mass index (BMI). These results suggest that delaying assisted conception to make changes to lifestyle is unlikely to enhance conception.

DESIGN: Unmatched case-referent study with 939 cases and 1310 referents. Cases had a low-MSC relative to the time since last ejaculation ($<12 \times 10^6$ for 3 days of abstinence). Exposures included self-reported exposures to alcohol, tobacco, recreational drugs as well as occupational and other factors.

PARTICIPANTS AND SETTING: Eligible men, aged 18 or above, were part of a couple who had been attempting conception without success following at least 12 months of unprotected intercourse and also had no knowledge of any semen analysis. They were recruited from 14 fertility clinics across the UK during a 37-month period from 1 January 1999.

MAIN RESULTS AND THE ROLE OF CHANCE: Risk factors for low MSC, after adjustment for centre and confounding factors, included a history of testicular surgery [odds ratio = 2.39, 95% confidence interval (CI): 1.75, 3.28], being in manual work [odds ratio (OR) = 1.28, 95% CI: 1.07, 1.53] or not working (OR = 1.78, 95% CI: 1.22, 2.59) and having black ethnicity (OR = 1.99, 95% CI: 1.10, 3.63). Conversely, men who wore boxer shorts (OR = 0.76, 95% CI: 0.64, 0.92) or who had a previous conception (OR = 0.71, 95% CI: 0.60, 0.85) were less likely to be a case. No significant association was found with smoking and alcohol consumption, the use of recreational drugs, a high BMI or having a history of mumps or fever.

BIAS, CONFOUNDING AND OTHER REASONS FOR CAUTION: Data were collected blind to outcome, and exposure information should not have been subject to reporting bias. Among men attending the various clinics less than half met the study eligibility criteria and among those who did, two out of five were not recruited. It is not known whether any of those who refused to take part did so because they had a lifestyle they did not want subjected to investigation. Although the power of the study was sufficient to draw conclusions about common lifestyle choices, it cannot comment on exposures that are perhaps rare and poorly reported: the finding that use of street drugs was unrelated to low MSC cannot be assumed to apply to all such drugs and all patterns of use. The case definition did not consider sperm morphology or sperm DNA integrity.

GENERALIZABILITY TO OTHER POPULATIONS: All participating clinics saw patients at no cost (under the UK National Health Service) and the study population may differ from those in countries without such provision. Even within the UK, low-income couples may choose not to undertake any investigation believing that they would subsequently be unable to afford treatment.

[†] Participating centres are listed in Appendix.

STUDY FUNDING/COMPETING INTEREST(S): The study was funded by the UK Health and Safety Executive, the UK Department of Environment, Transport and the Regions, the UK Department of Health (grant code DoH 1216760) and the European Chemical Industry Council (grant code EMSG19). No competing interests declared.

Key words: infertility / male / semen analysis / lifestyle / case-referent studies

Introduction

Reviews of male subfertility often highlight how aspects of men's adult lifestyle can significantly increase their risk of subfertility (Sadeu *et al.*, 2010; Li *et al.*, 2011). The American Urological Association found insufficient data on outcomes to support an evidence-based guideline but its best practice statement for the evaluation of the infertile male recommends reviewing lifestyle exposures (American Urological Association Education and Research, Inc., 2010). The UK National Institute for Clinical Excellence suggests that when giving advice to infertile men, clinicians consider their occupation and five potentially modifiable lifestyle factors that are thought to increase the risk of poor semen quality (National Collaborating Centre for Women's and Children's Health, 2004). These include excessive alcohol intake and cigarette smoking, the wearing of tight underwear, having a body mass index (BMI) of >29 and the use of recreational drugs (such as anabolic steroids and cocaine). However, the data underpinning much of this advice is often contradictory and consequently the strength of evidence is relatively weak (National Collaborating Centre for Women's and Children's Health, 2004).

Recently, there have been important insights into how maternal lifestyle choices around conception and while pregnant may affect future (adult) fertility of a male fetus, for example, prenatal exposure to gonadotoxic agents (e.g. cigarette smoking during pregnancy) can reduce the adult semen quality of any sons born (Ramlau *et al.*, 2007; Sharpe, 2010). However, our understanding of how the lifestyle choices of adult men themselves might impact on their fertility remains uncertain and often contradictory (Sharpe, 2010). Therefore, to increase our knowledge in this area we examined lifestyle data of men recruited to the large multicentre Chemical and Pregnancy Study (Chaps-uk; Cherry *et al.*, 2008) and used it to examine reportedly important (and potentially reversible) lifestyle risks for poor semen quality.

Methods

Design and recruitment

Chaps-uk was a multicentre case-referent study in which cases and referents were male patients identified at their first visit to either a fertility or gynaecology clinic, or to an andrology laboratory for semen analysis or when a first appointment was made for a semen analysis. The study design and methods have been described elsewhere (Clyma, 2004; Cherry *et al.*, 2008). Briefly, men aged 18 or above were recruited from 14 fertility clinics across the UK during a 37-month period from 1 January 1999. They were potentially eligible to take part in the study if they had been attempting conception without success following at least 12 months of unprotected intercourse, the standard clinical definition for couple infertility (Zegers-Hochschild *et al.*, 2009). They also had to have no knowledge of the result of any semen analysis (i.e. to have had no previous analysis or the results of an analysis were not yet communicated): this criterion was included to remove the possibility of bias in response to questions on exposures and risk factors. Only those assessed by the interviewer to be able to understand English were included. Men were excluded if they had a known medical condition which could be the cause of their infertility (e.g. genetic conditions such as cystic fibrosis), or if they had ever undergone treatment that could be a cause of their infertility (e.g. chemotherapy). Men in couples where the infertility problem was due to previous sterilization (vasectomy or tubal ligation) were excluded.

If the man was recruited he was asked to complete at home a brief questionnaire on job history, lifestyle and health factors and was requested to abstain from ejaculation for a period of 3–5 days (depending on the clinic) prior to the clinic visit. On presentation at the clinic for the first appointment following consent, the subject was interviewed to elaborate on existing information and to obtain additional information on the type of underwear and clothing worn by the patient, recreational drug use, fertility history, and, after December 1999, ethnic group, height and weight.

Collection and analysis of semen samples

Participants provided a semen sample for a diagnostic analysis as part of infertility investigations with their partner: this sample was also used for this study. The semen sample was collected into a standard plastic container and analysed according to a protocol based upon the techniques outlined by the World Health Organization (1999) as described previously (Cherry *et al.*, 2008).

The analyses were carried out in 12 andrology laboratories associated with 14 hospital clinics; each was a member of the semen analysis scheme of the UK National External Quality Assessment Service to provide external quality assurance. Sperm concentration was estimated at each site using a haemocytometer on a single dilution made in each laboratory at the time of semen analysis. Motility was captured on videotape using a computer out station commissioned for the study from Hobson Tracker Systems Limited, Sheffield, UK and returned to the central laboratory for analysis of sperm motility by computer-assisted sperm analysis (CASA). The proportion of motile sperm was calculated as the % of sperm moving forward at 5 μm/s or greater.

Case definition

The initial case definition, of $<12 \times 10^6$/ml progressively motile sperm, was based on earlier studies of solvents and male fertility (Cherry *et al.*, 2001, 2008). These were based on the WHO recommendations then in place for 'normal' sperm count (20×10^6/ml) and motility (60%; World Health Organization, 1980). Motile sperm concentration (MSC) was strongly related to the period of abstinence since last ejaculation and a moving cut-off was subsequently adopted (Cherry *et al.*, 2008) to ensure that the proportion defined as a case was the same in each abstinence group. In those with 3 day's abstinence, 41.8% had an MSC meeting the initial case definition. This percentage was used for the definition of a case in each abstinence group, resulting in a moving cut-off point on the MSC scale. The cut-off for those with 0–2, 4, 5 and 6 or more days abstinence was then <10.4, <13.9, <15.8 and $<17.0 \times 10^6$ MSC, respectively (Cherry *et al.*, 2008).

Factors examined for relation to MSC

All information was by self-report, with no attempt made within the study to confirm the accuracy of health reports, for example report of a fever, mumps or a pelvic ultrasound or X-ray (pelvic imaging) or to correct interpretations ('pelvic' investigations may have included examinations of the abdomen or lower back, for example). Events believed to indicate an irreversible risk such as surgery to the testes (usually for cryptorchidism) or pelvic imaging or mumps were included in the analysis if they had occurred at any point prior to semen collection. More transient factors (fever, use of tobacco, alcohol and/or street drugs) were coded positive if they occurred during the 3-month window prior to sample collection. Other factors were taken as reported by the subject at the time of the interview (previous conceptions, age of subject and partner, work status and usual type of underwear).

All demographic and lifestyle factors were categorized for analysis, either as a binary factor (ever surgery to the testes, usually wore boxer shorts) or using conventional breakpoints: WHO for BMI (World Health Organization, 2012), UK census for age and manual work (Office of Population Censuses and Surveys, 1990). For composite factors incorporating timing as well as occurrence, cut-points were taken that seemed biologically most plausible (mumps reported to have been in adolescence or later, fever lasting 14 days or more). Smoking tobacco and drinking alcohol were considered both as binary factors (ever/never within the 91 days prior to semen collection) and total consumption within that time window, grouped to reflect conventional boundaries for a man smoking or drinking throughout those 91 days (for example smoking <910 cigarettes over 91 days or drinking ≤ 130 units of alcohol over 13 weeks).

Statistical methods

The data were collected from 14 clinics and analysed in 12 laboratories in 11 regional centres. The clustering of the data within 11 regions was captured using a multilevel logistic regression model (gllamm in Stata 9) with centre-specific random intercepts. Such analysis was carried out first for each factor independently, then in a single model with all factors. Subjects with one or more missing values on any factor other than BMI or ethnicity were excluded from the analysis. For BMI and ethnicity, where some 10% were missing because of the late addition of the information, a 'missing' category was included in the effect estimation. A final model was constructed with only those factors that added significantly ($P < 0.05$) to the gllamm regression with allowance for clustering.

Power

The power of the study depends on the prevalence of each risk factor in the population. The illustration here is for smoking. In 2000, 35% of men aged 20–49 in Britain smoked (Rickards *et al.*, 2004). It was calculated that, with 2300 subjects and a 2:1 ratio of controls, and a true relative risk (RR) of 1.3 for smoking, the study would have 81% power to find a statistically significant result using a two-tailed test ($P < 0.05$).

Ethical approval

This study was approved by the MultiCentre Ethics Committee for the North West (Ref. No. MREC 98/8/73) with subsequent site approval given by Local Research Ethics Committees.

Results

Of the 11 680 men with an appointment for fertility investigations, 4257 (36.5%) men were eligible for the study. Two thousand two hundred and forty-nine were successfully recruited, took part in an interview and gave a semen sample. Of the remaining men, 572 did not attend their clinic appointment, 162 refused because they did not have the time (citing parking meters, for example), 30 refused because they felt that the study would be too stressful and 1168 refused without a reason. Semen analysis results were not obtained from 76 subjects who completed the rest of the study and these men have been excluded from the results reported here. Of those recruited, 68% were approached at a fertility or gynaecology clinic and 32% at an andrology laboratory. The initial case definition of $<12 \times 10^6$/ml progressively motile sperm was met by 871 (38.7%) of these 2249 subjects. However, applying the revised threshold adopted for the study (reflecting abstinence before sample), 939 of the 2249 men were classified as cases.

In the initial analysis, after adjustment for centre, MSC was associated with nine factors. These included six non-modifiable factors (Table I) associated with the personal characteristics of the man (ethnicity, previous conception), their health (having testes surgery or pelvic imaging, having mumps at the age of 13 or older, having a fever lasting 2 weeks or more in the 3 months prior to the semen analysis). The man's age and their partner's age were not significantly associated with risk of being a case.

There were three modifiable lifestyle and occupational exposures associated with low MSC namely being in manual work or not working, not wearing boxer shorts and not drinking alcohol in the 3 months prior to semen analysis (Table II). Other potential modifiable risk factors such as BMI, cigarette smoking, use of street drugs or cannabis were not associated with risk.

Multivariable analyses

The associations between the nine putative risk factors (Tables I and II) and the risk of being a case were examined when all factors were in the same model. Only five factors then remained significantly associated with MSC, namely ethnicity, testes surgery, previous conception, manual work and the wearing of boxer shorts. Table III shows the risk associated with each individual factor after adjustment for centre and the other four variables in the multivariable model. In this analysis men of black ethnicity remained more likely than Caucasian men to be a case [odds ratio (OR) = 1.99, 95% confidence interval (CI): 1.10, 3.63]. Similarly men who had undergone testes surgery were more likely to have low MSC (OR = 2.39, 95% CI: 1.75, 3.28) as were men who were in manual work (OR = 1.28, 95% CI: 1.07, 1.53) or were not working (OR = 1.78, 95% CI: 1.22, 2.59). In contrast men who wore boxer shorts (OR = 0.76, 95% CI: 0.64, 0.92) or who had a previous conception (OR = 0.71, 95% CI: 0.60, 0.85) were less likely to be a case.

After adjustment for these five factors, the risk of being a case was reduced (but still >1.0) in those men who had had pelvic imaging (OR = 1.19, 95% CI: 0.97, 1.46), mumps at 13 or older (OR = 1.71, 95% CI: 0.94, 3.13) or a fever lasting for 2 weeks or more in the 3 months before semen analysis (OR = 1.34, 95% CI: 0.98, 2.41).

The reduced risk associated with consuming alcohol in the 3 months prior to semen analysis was no longer significant after adjustment for centre and the other five risk factors (OR = 0.85, 95% CI: 0.69, 1.05; Table IV). However, in those men who had consumed over 3 months between 131 and 273 units of alcohol (i.e. >10 and

Table I Non-modifiable characteristics of cases and referents.

Risk factor	Value	Case (%) (n = 939)	Referent (%) (n = 1310)	OR (95% CI)[a]
Age subject	18–30	295 (31.4)	425 (32.4)	1
	31–40	535 (57.0)	737 (56.3)	1.04 (0.87–1.25)
	41–50	98 (10.4)	138 (10.5)	1.02 (0.76–1.38)
	51+	11 (1.2)	10 (0.8)	1.61 (0.67–3.85)
Ethnic group	White	703 (74.9)	1007 (76.9)	1
	Black	27 (2.9)	21 (1.6)	1.84 (1.03–3.29)
	Asian	41 (4.4)	52 (4.0)	1.14 (0.75–1.74)
	Other	18 (1.9)	23 (1.8)	1.13 (0.60–2.11)
	Not asked	150 (16.0)	207 (15.8)	1.03 (0.82–1.30)
Previous conception	No	596 (63.5)	747 (57.0)	1
	Yes	340 (36.2)	560 (42.7)	0.76 (0.64–0.90)
	Unknown	3 (0.3)	3 (0.2)	
Testes surgery	No	821 (87.4)	1231 (94.0)	1
	Yes	110 (11.7)	70 (5.3)	2.35 (1.72–3.22)
	Unknown	8 (0.9)	9 (0.7)	
Pelvic imaging	No	704 (75.0)	1029 (78.5)	1
	Yes	224 (23.9)	268 (20.5)	1.22 (1.00–1.49)
	Unknown	11 (1.2)	13 (1.0)	
Mumps	No	325 (34.6)	466 (35.6)	1
	Yes, not >13 years old	265 (28.2)	364 (27.8)	1.04 (0.84–1.29)
	Yes, >13 years old	27 (2.9)	21 (1.6)	1.85 (1.03–3.34)
	Yes, age unknown	322 (34.3)	459 (35.0)	1.00 (0.82–1.23)
Fever in 3 months before	No	774 (82.4)	1098 (83.8)	1
	Yes, lasting <2 weeks	112 (11.9)	160 (12.2)	1.00 (0.77–1.29)
	Yes, lasting ≥2 weeks	43 (4.6)	38 (3.0)	1.56 (1.00–2.43)
	Yes, length unknown	10 (1.1)	13 (1.0)	1.10 (0.48–2.52)
Age partner	18–30	458 (48.8)	604 (46.1)	1
	31–40	441 (47.0)	647 (49.4)	0.90 (0.76–1.07)
	41+	32 (3.4)	52 (3.4)	0.81 (0.51–1.28)
	Unknown	8 (0.9)	7 (0.5)	

[a]Adjusted for centre.

≤21 units/week) there remained evidence of a protective effect (OR = 0.78, 95% CI: 0.61, 1.00; Table IV).

Smoking within the 3 months prior to semen analysis was not associated with being a case (Table IV): 36.2% of cases and 35.3% of referents had smoked during that time period (adjusted OR = 0.96, 0.80, 1.16). There was no evidence of a dose–response relationship and those men who smoked <910 cigarettes were less likely to be a case (OR = 0.71, 95% CI: 0.51, 0.99). Although there was some suggestion that those who smoked between 910 and 1800 cigarettes during this time period were somewhat more likely to be a case (OR = 1.20, 95% CI: 0.94, 1.55), this trend was not seen in the heaviest smokers (OR = 0.99, 95% CI: 0.70, 1.19).

Discussion

The study identifies, using a case-referent analysis, demographic and social factors associated with low MSC in the male partners of couples presenting for fertility investigations. Only five factors were related to case status after adjustment for potential confounding factors, with men more likely to have low MSC if they had had a history of surgery to the testes; were of black ethnic origin or were in manual work (or not working at all). Conversely, men were less likely to be a case, and have higher MSC, if they wore boxer shorts or had a history of a previous conception.

Some of the identified risk factors are well recognized but others less so. We did not identify the reason for testicular surgery but undescended testicles, the most likely reason, is associated with reduced fertility (Virtanen *et al.*, 2007). A higher risk for those in manual work has been reported in a previous analysis of these data which is likely to be due, in part, to increased exposure to glycol ethers and other toxicants (Cherry *et al.*, 2008). However, after allowance for such exposures men in manual work remained at increased risk of low-motile sperm count, suggesting that there are other differences between manual and non-manual work that were not identified in this study. We were unable to identify any informative subgroups by analysis of occupational and industry codes. The high risk in those not working (students and the unemployed) is also not readily explained.

The lower risk of being a case in those usually wearing boxer shorts is consistent with some (Tiemessen *et al.*, 1996) but not all previous studies (Munkelwitz and Gilbert, 1998). The increased risk in men of black ethnicity is of interest, but the numbers are small and hence no strong conclusion can be reached. We find little evidence to suggest that smoking,

Table II Modifiable characteristics of cases and referents.

Risk factor	Value	Case (%) (n = 939)	Referent (%) (n = 1310)	OR (95% CI)[a]
Body mass index (BMI)	18.5–22.99 (low normal)	170 (18.1)	214 (16.3)	1
	23–24.99 (high normal)	171 (18.2)	234 (17.9)	0.92 (0.69–1.22)
	25–29.99 (overweight)	334 (35.6)	502 (38.3)	0.84 (0.65–1.07)
	>30 (obese)	94 (10.0)	134 (10.2)	0.88 (0.63–1.22)
	<18.5 (underweight)	8 (0.9)	6 (0.5)	1.71 (0.58–5.03)
	Not asked/unknown	162 (17.3)	220 (16.8)	0.92 (0.69–1.23)
Manual work	No	401 (42.7)	649 (49.5)	1
	Yes	473 (50.4)	585 (45.4)	1.29 (1.08–1.53)
	Not working	65 (6.9)	66 (5.0)	1.60 (1.11–2.30)
Boxer shorts (usually)	No	325 (34.6)	391 (29.8)	1
	Yes	608 (64.7)	914 (69.8)	0.80 (0.67–0.96)
	Unknown	6 (0.6)	5 (0.4)	
Alcohol in 3 months before	No	216 (23.0)	248 (18.9)	1
	Yes	723 (77.0)	1062 (81.1)	0.78 (0.64–0.96)
Cigarettes in 3 months before	No	599 (63.8)	848 (64.7)	1
	Yes	340 (36.2)	462 (35.3)	1.04 (0.87–1.24)
Street drugs	No	851 (90.6)	1182 (90.2)	1
	Yes	85 (9.1)	124 (9.5)	0.95 (0.71–1.27)
	Unknown	3 (0.3)	4 (0.3)	
Cannabis	No	862 (91.8)	1194 (91.1)	1
	Yes	74 (7.9)	108 (8.2)	0.95 (0.70–1.29)
	Unknown	3 (0.3)	8 (0.6)	

[a]Adjusted for centre.

Table III Multivariable analysis of risk factors for poor semen quality (n = 2195).

Risk factor	Value	OR (95% CI)[a]
Ethnic group	White	1.0
	Black	1.99 (1.10–3.63)
	Asian	1.06 (0.69–1.64)
	Other	1.11 (0.59–2.10)
	Not asked	0.99 (0.78–1.26)
Testes surgery	No	1.0
	Yes	2.39 (1.75–3.28)
Manual work	No	1.0
	Yes	1.28 (1.07–1.53)
	Not working	1.78 (1.22–2.59)
Boxer shorts (usually)	No	1.0
	Yes	0.76 (0.64–0.92)
Previous conception	No	1
	Yes	0.71 (0.60–0.85)

[a]Adjusted for centre and other risk factors shown.

excessive alcohol or recreational drug use or a high BMI alter MSC, although it remains possible that they affect fertility through some other mechanism (e.g. a high BMI may alter male fertility through changes in sex hormones; Hammoud *et al.*, 2008). A very low BMI appeared to carry risk as suggested previously (Jensen *et al.*, 2004) but the small numbers in this study provide only weak supportive evidence.

The absence of effect of tobacco on MSC is consistent with some (Trummer *et al.*, 2002; Swan *et al.*, 2003; Li *et al.*, 2009) but not all (Vine *et al.*, 1996; Eskenazi *et al.*, 2003) previous studies. Reviews also reflect this uncertainty in that some (Marinelli *et al.*, 2004; Sharpe, 2010) but not all (Vine, 1996; Li *et al.*, 2011) reviews suggest that smoking has, at most, a limited effect on semen quality. This inconsistency may, in part, be explained by differences in data analysis in that few published studies adjust for confounders such as sexual abstinence. In the present study, the OR for the heaviest smokers suggested that the risk was effectively identical to those who did not smoke. Our results do not rule out small changes within the normal MSC range but the biological significance of such a reduction is uncertain and this is reflected in a recent statement from the Practice Committee of the American Society for Reproductive Medicine that the 'effect of smoking on male fertility is [more] difficult to discern' (Practice Committee of American Society for Reproductive Medicine, 2008b).

In the univariate analysis there was no suggestion of any reduction in MSC by drinking alcohol, with drinkers if anything being protected, a finding that has previously been reported (Marinelli *et al.*, 2004). In the full model, the ORs were all <1.00 (compared with those not reporting any alcohol use in the 91 days before semen analysis). In a study of non-smoking, non-drug using male alcoholics who had been drinking heavily for at least 5 days a week for at least a year and had been admitted to an addiction treatment centre, marked differences in sperm characteristics were found when compared with never drinking volunteers, suggesting prolonged and heavy drinking may affect fertility (Muthusami and Chinnaswamy,

Table IV Alcohol and smoking and alcohol by case-referent status: total consumption in 3 months before semen sample collected.

Exposure	Level	Cases (%)	Referents (%)	OR_{adj} (95% CI)[a]
Alcohol (units)	None	216 (23.0)	248 (18.9)	1
	Any	723 (77.0)	1062 (81.1)	0.85 (0.69–1.05)[b]
	1 < 131	276 (29.4)	370 (28.2)	0.91 (0.71–1.17)
	131 ≤ 273	243 (25.9)	393 (30.0)	0.78 (0.61–1.00)
	274 ≤ 455	128 (13.6)	187 (14.3)	0.87 (0.65–1.18)
	>456	70 (7.5)	104 (7.9)	0.81 (0.57–1.17)
Cigarettes (number)	None	599 (63.8)	848 (64.7)	1
	Any	340 (36.2)	462 (35.3)	0.96 (0.80–1.16)[c]
	1 < 910	63 (6.7)	116 (8.9)	0.71 (0.51–0.99)
	910 ≤ 1800	154 (16.4)	169 (12.9)	1.20 (0.94–1.55)
	1820 < 6400	120 (12.8)	172 (13.1)	0.99 (0.70–1.19)

[a]Adjusted for centre, previous conception, manual work/not working, boxer shorts, testes surgery and ethnicity.
[b]Versus those who did not drink alcohol.
[c]Versus those did not smoke.

2005). Nevertheless, the mean MSC in these alcoholics was higher (15.8 million) than the cut-off of 12 million used in this paper.

In considering the potential implications of our findings, it is appropriate to consider first the methodological strengths of the design. Importantly, these data were collected from men in couples all of whom had failed to conceive after 12 months and from men who, at the time of the interview, did not know the result of their semen analysis. The data were therefore collected blind to outcome, and should not have been biased (with, for example, men with poor semen being more inclined to deny cigarette use than someone whose semen analysis appeared normal). Thus, although we have not validated reports (by measuring urinary cotinine, for example) and we must assume that some degree of misclassification exists, we can reasonably conclude that our findings do not result from differential misclassification. A second strength arises from the central assessment by CASA of semen samples in a single laboratory: there was no opportunity for collaboration centre errors arising from measurement techniques that have been well described (Pacey, 2006). Third, this large, multicentre, study has the statistical power to uncover both small and large effects. The power of the study to detect an RR of 1.3, based on the observed numbers of cases and controls, was 84%, close to the anticipated figure and we are confident that, for example, cigarette smoking in the 3 months before the semen sample had little detrimental effect on MSC.

On the other hand, the study does have weaknesses. First, there may have been over-matching—the choice of a referent who was attending a fertility clinic will reduce the study power to identify exposures if these are also related to infertility by mechanisms other than MSC (e.g. effects on morphology). To investigate this, we carried out a sub-analysis ($n = 915$) of those with information on tubal occlusion in the female partner (and where the fertility of the male may be assumed to be close to that of the normal population). The results of this sensitivity analysis did not alter conclusions from the study: in the stratum in which the partner's Fallopian tubes were not fully patent ($n = 212$) smoking in the last 3 months, for example, was significantly less common in cases (28.2%) than in referents (47.8%). Second, the men in this study may not be representative of all men in couples with fertility problems. Although the participating couples were all being investigated with no financial cost, many low-income couples may choose not to undertake any investigation believing that they would subsequently be unable to afford treatment. Even among those attending the various clinics less than half met the study eligibility criteria and among those who did, two out of five were not recruited. We do not know how many of those who refused did so because they had a lifestyle they did not want subjected to investigation. This may have been rare, but we cannot dismiss it. Further, although the power of the study was sufficient to draw conclusions about common lifestyle choices it cannot comment on exposures that are perhaps rare and poorly reported: the finding that use of street drugs was unrelated to low MSC cannot be assumed to apply to all such drugs and all patterns of use. We cannot be certain either that all street drug use was reported. We also cannot rule out the possibility that men may have underestimated their exposure to certain lifestyle factors as they were aware that as a couple they were experiencing infertility. This (if present) would be more likely to impact on the power of the study than introduce bias; any underestimation would be unlikely to differ between cases and controls as case status was unknown before the questionnaire data were collected. The study would have been strengthened if there had been funds for exposure biomarkers (including those for tobacco, alcohol and drug use). The use of a moving cut-off for motile sperm count was introduced after the study was completed but uninfluenced by the findings: the results presented here are very similar indeed to those using the original case definition. Finally, our case definition did not consider sperm morphology which has been shown to be independently linked to the probability of conception (Guzick *et al.*, 2001) or sperm DNA integrity which is of increasing interest (Pacey, 2010), although there is currently no consensus about its significance or standardized measurements on how it might be assessed (Practice Committee of American Society for Reproductive Medicine, 2008a; Barratt *et al.*, 2010). The extent to

which these are altered by the risk factors described here is worthy of further study.

The study has identified few modifiable factors other than wearing loose underwear and avoiding exposures in certain manual jobs (Cherry *et al.*, 2008). It may be questioned whether another research design (such as collecting semen samples from random volunteers) with other outcome measures (perhaps the product of count and percent motility used as a transformed continuous variable) might have demonstrated small effects with significance at the population level more readily than the design adopted here. If the question of interest is however the couple's inability to conceive then small changes in MSC may be of very little significance. We know that time to conception is only adversely affected when MSC drops $<25-30 \times 10^6$ per ml (Larsen *et al.*, 2000). The design adopted here is directly relevant to addressing modifiable lifestyle factors that might help couples facing a diagnosis of infertility. Whilst the message of 'no smoking, no alcohol and no street drugs' should be offered as good health advice, our study shows that common lifestyle choices, other than wearing tight underwear, make little contribution to MSC and that delaying assisted conception to make poorly evidenced changes to lifestyle is unlikely to enhance conception and may indeed be prejudicial in couples with little time to lose.

Acknowledgements

The study was designed and initiated with the support of Professor Ian Cooke of the University of Sheffield. The research was made possible by the input from many, in Manchester, Sheffield and in each of the centres forming Chaps-uk. Clinicians and scientists at the participating centres include Sheena Lewis, Neil McClure (Belfast), Masoud Afnan (Birmingham), Chris Ford (Bristol), Lucas Klentzeris, Linda Gregory (Cardiff), Stewart Irvine (Edinburgh), Iwan Lewis-Jones (Liverpool), Brian Leiberman, David Poulson and Paul Bishop (Manchester), Adrian Lower and Melanie Davies (London), Alison Murdoch (Newcastle), Jason Kasraie (Shrewsbury) and William Ledger (Sheffield). The research coordinators and administrative staff included, as well as authors J.-A.C. and H.B., Yvonne King, Priscilla Appelbe, Chris Pappas, Deborah Saxton, Ana Roby and Jeanette Tenggren. Technical staff in Manchester and Sheffield included Mark Carus, Kelly Morris, Louise Reeve, Linda Street, Andrew Thomas and Hayley Willis. Each clinical and andrology centre had a team including clinicians, nursing and technical staff, some 70 between the centres and we are greatly indebted for their contributions.

Authors' roles

A.C.P., A.A.P. and N.C. drafted the manuscript; N.C., R.M., A.C.P., A.A.P. discussed and performed the statistical analysis. J.A.C. and H.B. co-ordinated the study and took care of communication and distribution of study materials to group members. Data management was done by J.A.C., H.B., N.C. and H.M. All members of the co-ordinating group contributed to the collection of data for the study and discussions on the design, conduct and interpretation of the results. A.C.P. and N.C. are guarantors.

Funding

The study was funded by the UK Health and Safety Executive, the UK Department of Environment, Transport and the Regions, the UK Department of Health (grant code DoH 1216760) and the European Chemical Industry Council (grant code EMSG19).

Conflict of interest

None declared.

References

American Urological Association Education and Research, Inc. The optimal evaluation of the infertile male: AUA best practice statement, 2010. Linthicum, MD: American Urological Association, 2010. http://www.auanet.org/content/media/optimalevaluation2010.pdf.

Barratt CL, Aitken RJ, Björndahl L, Carrell DT, de Boer P, Kvist U, Lewis SE, Perreault SD, Perry MJ, Ramos L *et al.* Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications—a position report. *Hum Reprod* 2010;**25**:824–838.

Cherry NM, Labreche F, Collins J, Tulandi T. Occupational exposure to solvents and male infertility. *Occup Environ Med* 2001;**58**:635–640.

Cherry N, Moore H, McNamee R, Pacey A, Burgess G, Clyma JA, Dippnall M, Baillie H, Povey A. Occupation and male infertility: glycol ethers and other exposures. *Occup Environ Med* 2008;**65**:708–714.

Clyma JA. Lifestyle and health factors and the risk of male infertility. Ph.D. Thesis. University of Manchester, 2004.

Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D. The association of age and semen quality in healthy men. *Hum Reprod* 2003;**18**:447–454.

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA *et al.* Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001;**345**:1388–1393.

Hammoud AO, Gibson M, Peterson CM, Meikle AW, Carrell DT. Impact of male obesity on infertility: a critical review of the current literature. *Fertil Steril* 2008;**90**:897–904.

Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE. Body mass index in relation to semen quality and reproductive hormones among 1558 Danish men. *Fertil Steril* 2004;**82**:863–870.

Larsen L, Scheike T, Jensen TK, Bonde JP, Ernst E, Hjollund NH, Zhou Y, Skakkebaek NE, Giwercman A. Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. The Danish First Pregnancy Planner Study Team. *Hum Reprod* 2000;**15**:1562–1567.

Li Y, Lin H, Ma M, Li L, Cai M, Zhou N, Han X, Bao H, Huang L, Zhu C *et al.* Semen quality of 1346 healthy men, results from the Chongqing area of southwest China. *Hum Reprod* 2009;**24**:459–469.

Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. *Fertil Steril* 2011;**95**:116–123.

Marinelli D, Gaspari L, Pedotti P, Taioli E. Mini-review of studies on the effect of smoking and drinking habits on semen parameters. *Int J Hyg Environ Health* 2004;**207**:185–192.

Munkelwitz R, Gilbert BR. Are boxer shorts really better? A critical analysis of the role of underwear type in male subfertility. *J Urol* 1998;**160**:1329–1333.

Muthusami KR, Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertil Steril* 2005;**84**:919–924.

National Collaborating Centre for Women's and Children's Health (NCCWCH). *Fertility: Assessment and Treatment for People with Fertility Problems*. London: National Institute for Clinical Effectiveness, Royal College of Obstetricians and Gynaecologists Press, 2004.

Office of Population Censuses and Surveys. Standard occupational classification. London: HMSO, 1990.

Pacey AA. Is quality assurance in semen analysis still really necessary? A view from the andrology laboratory. *Hum Reprod* 2006;**21**:1105–1109.

Pacey AA. Environmental and lifestyle factors associated with sperm DNA damage. *Hum Fertil (Camb)* 2010;**13**:189–193.

Practice Committee of American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing. *Fertil Steril* 2008a;**90**(5 Suppl.):S178–180.

Practice Committee of American Society for Reproductive Medicine. Smoking and infertility. *Fertil Steril* 2008b;**90**(5 Suppl.):S254–259.

Ramlau-Hansen CH, Thulstrup AM, Storgaard L, Toft G, Olsen J, Bonde JP. Is prenatal exposure to tobacco smoking a cause of poor semen quality? A follow-up study? *Am J Epidemiol* 2007;**165**:1372–1379.

Rickards L, Fox K, Roberts C, Fletcher L, Goddard E. Living in Britain. No 31. Results from the 2002 General Household Survey, 2004. http://www.ons.gov.uk/ons/dcp14763_170919.pdf.

Sadeu JC, Hughes CL, Agarwal S, Foster WG. Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: reproductive health consequences and clinical implications. *Crit Rev Toxicol* 2010;**40**:633–652.

Sharpe RM. Environmental/lifestyle effects on spermatogenesis. *Phil Trans R Soc B* 2010;**365**:1697–1712.

Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW; Study For Future Families Research Group. Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect* 2003;**111**:414–420.

Tiemessen CH, Evers JL, Bots RS. Tight-fitting underwear and sperm quality. *Lancet* 1996;**347**:1844–1845.

Trummer H, Habermann H, Haas J, Pummer K. The impact of cigarette smoking on human semen parameters and hormones. *Hum Reprod* 2002;**17**:1554–1559.

Vine MF. Smoking and male reproduction: a review. *Int J Androl* 1996;**19**:323–337.

Vine MF, Tse CK, Hu P, Truong KY. Cigarette smoking and semen quality. *Fertil Steril* 1996;**65**:835–842.

Virtanen HE, Bjerknes R, Cortes D, Jørgensen N, Rajpert-De Meyts E, Thorsson AV, Thorup J, Main KM. Cryptorchidism: classification, prevalence and long-term consequences. *Acta Paediatr* 2007;**96**:611–616.

World Health Organization. *Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*, 1st edn. Singapore: Press Concern, 1980.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 4th edn. Cambridge, UK: Cambridge University Press, 1999.

World Health Organization. Global database on body mass index, 2012. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html.

Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, van der Poel S; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Hum Reprod* 2009;**24**:2683–2687.

Appendix

Participating centres were Department of Obstetrics and Gynaecology, Queens University, Belfast; Assisted Conception Unit, Birmingham Women's Hospital; Division of Obstetrics and Gynaecology, St Michael's Hospital, Bristol; Directorate of Women's Health, Southmead Hospital, Bristol; Cardiff Assisted Reproduction Unit, University of Wales; MRC Reproductive Biology Unit, Edinburgh; Reproductive Medicine Unit, Liverpool Women's Hospital; St Bartholomew's Hospital, London; Department of Obstetrics and Gynaecology, Royal Free and University College, London; Department of Reproductive Medicine, St Mary's Hospital, Manchester; IVF/Immunology Laboratory, Hope Hospital, Salford; Department of Histopathology, Wythenshawe Hospital, Manchester; International Centre for Life, Newcastle; Department of Obstetrics and Gynaecology, Jessop Hospital for Women, Sheffield; Shropshire and Mid-Wales Fertility Centre, Royal Shrewsbury NHS Trust.