Modifiable and non-modifiable risk factors for poor sperm morphology

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STUDY QUESTION: Are common lifestyle factors associated with poor sperm morphology?

SUMMARY ANSWER: Common lifestyle choices make little contribution to the risk of poor sperm morphology.

WHAT IS KNOWN ALREADY: Although many studies have claimed that men's lifestyle can affect sperm morphology, the evidence is weak with studies often underpowered and poorly controlled.

STUDY DESIGN, SIZE, DURATION: Unmatched case-referent study with 318 cases and 1652 referents. Cases had poor sperm morphology ($<$4% normal forms based on 200 sperm assessed). Exposures included self-reported exposures to alcohol, tobacco, recreational drugs as well as occupational and other factors.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eligible men, aged 18 years 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 before being enrolled. 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 poor sperm morphology, after adjustment for centre and other risk factors, included: (i) sample production in summer [odds ratio (OR) = 1.99, 95% confidence interval (CI) 1.43–2.72]; and (ii) use of cannabis in the 3 months prior to sample collection in men aged ≤30 years (OR = 1.94, 95% CI 1.05–3.60). Men who produced a sample after 6 days abstinence were less likely to be a case (OR = 0.64, 95% CI 0.43–0.95). No significant association was found with body mass index, type of underwear, smoking or alcohol consumption or having a history of mumps. This suggests that an individual's lifestyle has very little impact on sperm morphology and that delaying assisted conception to make changes to lifestyle is unlikely to enhance conception.

LIMITATIONS, REASONS FOR CAUTION: Data were collected blind to outcome and so exposure information should not have been subject to reporting bias. Less than half the men attending the various clinics met the study eligibility criteria and among those who did, two out of five did not participate. It is not known whether any of those who refused to take part did so because they had a lifestyle which they did not want subjected to investigation. Although the power of the study was sufficient to draw conclusions about common lifestyle choices, this is not the case for exposures that were rare or poorly reported.

WIDER IMPLICATIONS OF THE FINDINGS: 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. Since a computer performed the measurements of sperm morphology, these results may not be comparable with studies where sperm morphology was assessed by other methods.

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Introduction

The assessment of sperm morphology during semen analysis is an important part of male fertility assessment (WHO, 2010). The size and shape of the sperm head has a major impact on sperm hydrodynamic efficiency (Gillies et al., 2009). Only sperm with so-called 'ideal' or 'normal' morphology are thought to be able to pass through cervical mucus and navigate other barriers of the female reproductive tract (Suarez and Pacey, 2006) as well as tether and bind to the zona pellucida of the egg (Menkveld et al., 1991; Liu and Baker, 1992). Sperm that retain excess residual cytoplasm during spermatogenesis and epididymal transit are more likely to produce higher levels of reactive oxygen species (Rengan et al., 2012), which can damage other sperm and contribute to additional aspects of sperm dysfunction such as poor motility or increased levels of DNA damage. Poor morphology is also thought to be associated with increased DNA fragmentation, chromosomal abnormalities, poor chromatin packaging and increased levels of sperm aneuploidy (WHO, 2010). Therefore, sperm morphology could be regarded as a surrogate marker for the quality of spermatogenesis.

In comparison with other measures of semen analysis (e.g. sperm concentration and sperm motility), the impact of lifestyle on sperm morphology has been poorly explored. For example, in the review by Sharpe (2010) and the clinical guidelines by the UK National Institute for Clinical Excellence (National Collaborating Centre for Women's and Children's Health, 2004; 2013) the effect of lifestyle factors on sperm morphology is hardly mentioned. Recent papers have examined the relationship between body mass index (BMI) (Macdonald et al., 2013; Eisenberg et al., 2014) and cigarette smoking (Jeng et al., 2014) and poor sperm morphology, but with inconclusive results.

As part of a large multi-centre study to examine risk factors for poor semen quality in the UK, we recruited 2249 men. This data set has so far been used to examine risk factors for low motile sperm concentration associated with chemical exposure in the work place (Cherry et al., 2008), exposure to chlorination by-products in tap water (Iszatt et al., 2013) as well as common modifiable and non-modifiable lifestyle factors (Povey et al., 2012). This latter study found relatively few identifiable risk factors associated with men's lifestyle, somewhat contrary to many clinical guidelines (e.g. National Collaborating Centre for Women's and Children's Health, 2004; 2013). In this paper, we turn our attention to sperm morphology and use the same data set to examine whether the size and shape of sperm is affected by common lifestyle factors.

Methods

Design and recruitment

CHAPS-UK was a multi-centre 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 (Cherry et al., 2008; Povey et al., 2012). Men aged 18 years 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. 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 (genetic conditions such as cystic fibrosis or Kartagener's syndrome), or if they had ever undergone treatment that could be a cause of their infertility (e.g. chemotherapy, radiotherapy or immunosuppressive therapy). Men in couples where the difficulty with conception resulted from previous sterilization (vasectomy or tubal ligation) were also excluded.

If the man agreed to take part 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 by a research nurse to elaborate on the information contained in the questionnaire and to obtain additional information on the type of undergarments and clothing worn by the patient, recreational drug use, fertility history and, after December 1999, ethnic group and height and weight for the calculation of BMI.

Collection and analysis of semen samples

Men provided a semen sample as part of infertility investigations with their partner. This sample was also used for the study reported here. 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 (WHO, 1999), as described previously (Cherry et al., 2008; Povey et al., 2012). Morphology slides were prepared and fixed at each centre and mailed to the study laboratory in Sheffield where one slide from each subject was stained by the Papanicolaou method and 200 sperm assessed using as Computer Aided Sperm Morphometric Assessment (CASMA) system developed by Hobson Tracker Systems (Sheffield, UK). The machine was programmed to recognize as 'normal' stained sperm heads which fit to the dimensions given in WHO (1999): a length of 4.0–5.0 μm and a width of 2.5–3.5 μm, with a length-to-width ratio between 1.50 and 1.75. Each participating site was a member of the UK semen quality assessment scheme (United Kingdom National External Quality Assessment Service (UK-NEQAS) and CASMA performance in Sheffield was checked against UK-NEQAS target values throughout the study.

Case and referent definition for analysis of morphology

Following WHO (2010) guidelines, subjects were considered a case if, by the WHO strict definition of normal morphology, <4% of the 200 sperm assessed were of normal form. Referents were all those whose morphology could be assessed and for whom at least 4% of sperm were of normal morphology. Any man whose semen sample resulted in a slide with <200 sperm was not assessed for morphology and is excluded from the analysis reported here.

Factors examined for relation to sperm morphology

All information was by self-report, with no attempt made to confirm the accuracy of health reports (for example, report of a fever, mumps or a pelvic ultrasound or X-ray) 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 ultrasound/X-ray (pelvic imaging) or mumps, were included in the analysis if they had occurred at any point prior to the semen sample. 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 enrolment (i.e. from 91 days before the semen sample was provided). Other factors were taken as reported by the subject at the time of the interview (abstinence, previous conceptions, age of subject and partner, work status, 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, 2012 for BMI; UK census for age and manual work (Office for 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/13 weeks prior to the semen sample) 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). Season was defined by month of semen sample collection as spring (March–May) summer (June–August), autumn (September–November) and winter (December–February).

The period of abstinence requested before giving the sample varied between clinics within the range of 3–5 days. Reported abstinence was grouped as ≤3, 4–5 and ≥6 days.

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 software) with centre-specific random intercepts. Such analysis was carried out first for each factor independently. 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 univariate gllamm regression. Each of the factors with $P \geq 0.05$ was then added to this model in turn to check whether negative confounding (such as a negative correlation between age and cannabis use) might be masking an effect that could be investigated further by stratification. In our previous analysis of motile sperm count we built days of abstinence into the definition of a case (Cherry et al., 2008; Povey et al., 2012). Here, however, there was no clear a priori evidence of how abstinence would affect morphology and it was decided to treat abstinence along with other modifiable factors.

Power

The power of the study depends on the prevalence of each risk factor in the population and the true relative risk (RR). We calculated the minimum RR the study could detect, with 80% power using a two-tailed test ($P < 0.05$), for a range of prevalences. With 2200 subjects and a 5:1 ratio of controls to cases, the minimum RR would be 1.40 for a factor with 35% prevalence; for example, in 2000, 35% of men aged 20–49 years smoked (Rickards et al., 2004). For prevalences of 25, 15 10 and 5%, the minimum RRs are 1.43, 1.53, 1.63 and 1.90.

Results

Of the 11 680 men with an appointment for fertility investigations, 4257 (36.5%) were eligible for the study. Of the total, 2249 were successfully recruited, took part in an interview and gave a semen sample. Of those recruited, 68% were approached at a fertility or gynaecology clinic and

32% at an andrology laboratory. Of the 2249 samples, 81 (3.6%) were found to have no sperm on the morphology slide, 47 (2.1%) slides had fewer than the 200 sperm required by WHO (1999) for robust analysis, 43 (1.9%) had slides that were contaminated or in some other way faulty and 2 (0.1%) slides were lost in transit. The proportion of morphologically normal forms could thus be assessed for 2076, of which 376 (18.1%) met the definition of a case.

Inspection of the proportion by the date the sample was collected indicated an unexpectedly high proportion of cases during the first 6 months of the study (January–June 1999), where, among 106 slides for which morphology could be assessed 58 (54.7%) met the case definition, compared with 318/1970 (16.1%) after this run-in period. No clear reason for this discrepancy could be determined and so the analysis reported here is restricted to samples collected and analysed after June 1999; that is 2136 samples, from which morphology could be determined for 1970.

Non-modifiable characteristics of the cases and referents are shown in Table I and modifiable characteristics in Table II, with odds ratios (OR) calculated, for each variable in turn, having adjusted for clustering within centre.

None of the non-modifiable characteristics in Table I was related to having <4% sperm with normal morphology (other than an improbable reduction in risk with >2 weeks fever which was interpreted as a chance effect). Among the modifiable characteristics shown in Table II, the use of cannabis in the previous 3 months was related to case status, with an OR of 1.55 [95% confidence interval (CI) 1.04–2.30] after allowing for clustering within centre. The use of cannabis was related to the age of the subject with 76/627 (12.1%) of men aged 18–30 years, 73/1124 (6.5%) aged 31–40 years and 13/209 (6.2%) aged >40 years reporting use: 10 men reporting use of a street drug did not specify a type and were excluded from the cannabis analysis. The wider category of street drugs was very highly correlated with cannabis use, and indeed few reported other substances. The season in which the sample was produced was also significant and samples collected in the summer had more abnormal forms (were more likely to meet the case definition) than those obtained at other times of year, with samples collected in winter being least likely to be classified as a case. The differences are quite marked, with 24.9% of summer samples indicating a case, compared with 13.1% of those collected in other seasons. Abstinence of 6 days or longer was associated with better morphology.

A multivariable model was constructed including the three factors associated ($P < 0.05$) with case status in a univariate model: season, cannabis use and abstinence. This is shown, overall and stratified by age group, in Table III. In the study group as a whole, having adjusted for other risk factors, samples produced in the summer remained more likely to be classified as a case (<4% normal morphology) and this pattern was seen in all age groups. Those giving a sample after at least 6 days abstinence were less likely to be a case, overall and in each age stratum, although this did not always reach significance. For cannabis use the effect was age related with the most marked increase in risk in men aged ≤30 years.

Finally, Table IV looks at the extent of alcohol and cigarette consumption in the 3 months before the semen sample was collected. Although there was a somewhat raised OR (1.51) for those reporting drinking >35 units of alcohol/week (>456 units over 13 weeks) this was not significant. There was less evidence of any association with number of cigarettes, with only slight and non-significant increase in risk (ORs around

Table I Non-modifiable characteristics of male cases (*n* = 318) and referents (*n* = 1652).

Risk factor	Value	Case		Referent		OR	95% CI[a]
		n	%	*n*	%		
Age of subject (years)	18–30	97	30.5	532	32.2	1	—
	31–40	185	58.2	945	57.2	1.09	0.84–1.43
	41–50	35	11.0	162	9.8	1.24	0.81–1.91
	51+	1	0.3	13	0.8	0.39	0.05–3.05
Ethnic Group	White	254	79.9	1331	80.6	1	—
	Black	10	3.1	30	1.8	1.49	0.71–3.16
	Asian	11	3.5	72	4.4	0.63	0.32–1.25
	Other	6	1.9	30	1.8	1.03	0.42–2.52
	Not asked	37	11.6	189	11.4	1.07	0.72–1.57
Previous conception	No	174	54.7	991	60.0	1	—
	Yes	142	44.7	658	39.8	1.22	0.95–1.56
	Unknown	2	0.6	3	0.2	—	—
Testes surgery	No	289	90.9	1527	92.4	1	—
	Yes	27	8.5	113	6.8	1.30	0.83–2.03
	Unknown	2	0.6	12	0.7	—	—
Pelvic imaging	No	237	74.5	1274	77.1	1	—
	Yes	76	23.9	364	22.0	1.11	0.84–1.48
	Unknown	5	1.6	14	0.8	—	—
Mumps	No	112	35.2	582	35.2	1	—
	Yes, not >13 years old	87	27.4	471	28.5	0.97	0.71–1.32
	Yes, >13 years old	7	2.2	34	2.1	1.06	0.46–2.47
	Don't know if had mumps	112	35.2	565	34.2	1.04	0.78–1.39
Fever in 3 months before	No	265	83.3	1377	83.4	1	—
	Yes, lasting < 2 weeks	46	14.5	199	12.0	1.20	0.85–1.70
	Yes, lasting ≥2 weeks	4	1.3	61	3.7	0.32	0.11–0.88
	Yes, length unknown	3	0.9	15	0.9	0.96	0.27–3.42
Age of partner (years)	18–30	149	46.9	774	46.9	1	—
	31–40	147	46.2	812	49.2	0.95	0.74–1.22
	41+	17	5.3	58	3.5	1.62	0.91–2.88
	Unknown	5	1.6	8	0.5	–	—

[a]Adjusted for clustering within centre. OR, odds ratio, CI, confidence interval.

1.20) for those smoking more than 10 cigarettes/day (>910 over 91 days).

None of the variables in Tables II and III other than season, cannabis use and abstinence (and fever >2 weeks as a protective effect) added to the all age model in Table III and addition of these three variables to alcohol and smoking analysis in Table IV did not reveal any effect masked by confounding.

Discussion

This study used a case-referent design to identify demographic and life-style factors associated with poor sperm morphology (teratozoospermia). Only three factors were independently related to case status after adjustment for clustering within centre: men who produced their sample in summer (June to August) and younger men who used cannabis in the 3 months prior to sample collection were more likely to have sperm morphology <4% normal. Men who produced a sample after at least 6 days abstinence were less likely to be a case (to have sperm morphology of ≥4%).

It is intriguing that the use of cannabis can have a measurable effect on sperm morphology and it seems likely that the more marked effect in younger men reflected greater quantity of consumption but as this study only recorded 'any use' this cannot be examined further here. In a small study of 16 men (Hembree *et al.*, 1978), it was suggested that sperm morphology could be compromised by cannabis use. However, no data were presented and no details were given about the method of sperm morphology assessment. Other studies on cannabis and male fertility have largely focused on the negative effects of the main psychoactive compound on sperm motility *in vitro* (Whan *et al.*, 2006) and it has been suggested that the cannabinoid system may play an important role in male reproduction (Rossato *et al.*, 2008). Interestingly, a recent study has found significant differences in the endocannabinoid system of sperm obtained from fertile and infertile men (Lewis *et al.*, 2012), which suggests that there may be differences in the way men respond to cannabis exposure. In relation to sperm morphology it has been noted that the cannabinoid receptor in mouse spermatids can influence chromatin remodelling (Chioccarelli *et al.*, 2010), thereby opening up a possible mechanism by which sperm morphology may be impaired in cannabis users.

In contrast, our finding that season of sample production significantly affected sperm morphology is consistent with some (Levitas *et al.*, 2013; Zhang *et al.*, 2013) but not all (Gyllenborg *et al.*, 1999; Jørgensen *et al.*, 2001; Carlsen *et al.*, 2004) previous findings. It is tempting to suggest

Table II Modifiable characteristics of cases (n = 318) and referents (n = 1652).

Risk factor	Value	Case		Referent		OR	95% CI[a]
		n	%	n	%		
BMI (kg/m^2)	18.5–22.99 (low normal)	63	19.8	289	17.5	1	—
	23–24.99 (high normal)	71	22.3	312	18.9	1.08	0.74–1.58
	25–29.99 (overweight)	106	33.3	658	39.8	0.75	0.53–1.06
	>30 (obese)	36	11.3	173	10.5	0.96	0.61–1.51
	<18.5 (underweight)	2	0.6	11	0.7	0.82	0.17–3.85
	Not asked/unknown	40	12.6	209	12.7	0.93	0.59–1.44
Manual work	No	143	45.0	806	48.8	1	—
	Yes	153	48.1	759	45.9	1.11	0.87–1.44
	Not working	22	6.9	87	5.3	1.46	0.88–2.43
Boxer shorts (usually)	No	97	30.5	528	32.0	1	–
	Yes	218	68.6	1117	67.6	1.07	0.82–1.39
	Unknown	3	0.9	7	0.4	—	—
Alcohol in 3 months before	No	57	17.9	336	20.3	1	—
	Yes	261	82.1	1316	79.7	1.23	0.90–1.68
Cigarettes in 3 months before	No	196	61.6	1083	65.6	1	—
	Yes	122	38.4	569	34.4	1.16	0.91–1.49
Street drugs	No	276	86.8	1502	90.9	1	—
	Yes	40	12.6	146	8.8	1.46	1.00–2.15
	Unknown	2	0.6	4	0.2	—	—
Cannabis	No	279	87.7	1519	91.9	1	—
	Yes	36	11.3	126	7.6	1.55	1.04–2.30
	Unknown	3	0.9	7	0.4	—	—
Season	Spring	68	21.4	408	24.7	1	—
	Summer	126	39.6	381	23.1	2.04	1.47–2.84
	Autumn	82	25.8	498	30.1	1.04	0.73–1.47
	Winter	42	13.2	365	22.1	0.73	0.48–1.10
Abstinence	≤3 days	163	51.3	774	46.9	1	—
	4–5 days	117	36.8	571	34.6	0.99	0.75–1.30
	≥6 days	38	11.9	307	18.6	0.57	0.39–0.85

[a]Adjusted for clustering within centre.

Table III Multivariable analysis of risk factors for normal sperm morphology <4%.

Risk factor	Value	All		Age (years)					
				18–30		31–40		>40	
		OR	95% CI[a]	OR	95% CI[a]	OR	95% CI[a]	OR	95% CI[a]
Season	Spring	1.0	—	1	—	1	—	1	—
	Summer	1.99	1.43–2.72	2.21	1.22–4.00	1.73	1.13–2.67	2.74	0.88–8.54
	Autumn	1.01	0.71–1.44	1.09	0.57–2.07	0.92	0.58–1.44	1.35	0.39–4.73
	Winter	0.74	0.49–1.13	0.64	0.28–1.43	0.68	0.40–1.18	1.31	0.39–4.42
Cannabis use	No	1	—	1	—	1	—	1	—
	Yes	1.45	0.97–2.17	1.94	1.05–3.60	1.35	0.63–1.29	0.97	0.20–4.76
Abstinence	≤3 days	1	—	1	—	1	—	1	—
	4–5 days	1.01	0.77–1.34	1.24	0.75–2.05	0.90	0.63–1.29	1.11	0.47–2.64
	≥6 days	0.64	0.43–0.95	0.77	0.38–1.64	0.54	0.32–0.92	0.81	0.28–2.32

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

that sperm morphology is reduced in the summer months because increased atmospheric temperature affected spermatogenesis in some way. However, sperm ejaculated in summer months would have started spermatogenesis in the cooler temperatures of spring, suggesting no simple relationship between morphology and atmospheric temperature. Moreover, in our univariate analysis we did not see any risk of poor morphology associated with fever or style of underwear, both of which would arguably increase scrotal temperature considerably at any time of

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

Exposure	Level	Case		Referent		OR	95% CI[a]
		n	%	n	%		
Alcohol (units)	None	57	17.9	336	20.3	1	—
	1 < 131	100	31.4	469	28.4	1.31	0.91–1.88
	131 ≤ 273	84	26.4	487	29.5	1.08	0.74–1.56
	274 ≤ 455	46	14.5	234	14.2	1.21	0.79–1.85
	≥456	29	9.1	117	7.1	1.51	0.91–2.49
	Amount unknown	2	0.6	9	0.5	—	—
Cigarettes (number)	None	196	61.6	1083	65.6	1	—
	1 < 910	25	7.9	137	8.3	0.97	0.61–1.53
	910 ≤ 1800	50	15.7	226	13.7	1.18	0.83–1.67
	1800 < 6400	44	13.8	201	12.2	1.22	0.85–1.75
	Amount unknown	3	0.9	5	0.3	—	—

[a]Adjusted for clustering within centre.

year. In our previous analysis of the risk of men providing a sample with a low motile sperm concentration, men who wore boxer shorts were significantly less likely to be a case (Povey et al., 2012) suggesting that scrotal heating through the wearing of restrictive underwear is sufficient to exert measurable testicular effects. Therefore, we question whether the seasonal effect on sperm morphology described here is mediated through changes in temperature.

Finally, the relationship between improved sperm morphology with 6 or more days of sexual abstinence is harder to explain. Neither of the studies by Carlsen et al. (2004) or DeJonge et al. (2004) could find any relationship between abstinence and sperm morphology; however, their studies were small (<50 subjects between them) and were arguably under-powered. In comparison, Levitas et al. (2005) examined the duration of sexual abstinence and semen quality of 9489 samples from 6008 men and generally found that morphology decreased with increasing sexual abstinence. We are unaware of any biological mechanism by which sperm size and shape could improve markedly during post-epididymal storage prior to ejaculation. Therefore, our findings, while strengthened by their consistency across age groups, may be down to chance or some as yet undefined aspect of male reproductive physiology.

No significant association was found between case status and the other medical and lifestyle factors examined in this study. In a recent study from New Zealand better sperm morphology was associated with increasing BMI but morphology was measured in only 330/511 cases and only 40% of the men, more obese than in the present study, were being investigated for infertility (MacDonald et al., 2013). In a study from the USA, with even greater obesity and low proportions with abnormal morphology, no relation was found between obesity and poor morphology (Eisenberg et al., 2014). With regard to cigarette smoking, most studies have suggested it has little effect on sperm morphology (Hoidas et al., 1985; Sergerie et al., 2000; Sepaniak et al., 2006), although they vary in their design and method of sperm morphology assessment. More recently, a study of 198 volunteers in Taiwan (Jeng et al., 2014) found that those smoking >10 cigarettes/day were less likely to have normal morphology (judged by WHO, 1999 criteria) although, as in the present study, smoking overall was not a risk factor.

To define normal sperm we chose to use the definition published in WHO (1999). Whilst other definitions of normal sperm exist, such as Strict Criteria (Menkveld et al., 1990), and alternative indices of sperm

morphology have been developed (Jouannet et al., 1988), we considered that the head size-shape definition of WHO (1999) was most appropriate for the analysis of our data set because it was the one used by Cooper et al., (2010) to define the <4% cut-off published in WHO (2010) and which we used to establish our case definition. Whilst the head size dimensions of normal sperm in WHO (1999) are slightly larger than the normal range now established for Papanicolaou stained sperm shown in WHO (2010), the analysis of morphology slides for this study was undertaken before their publication. The application of the WHO (2010) criteria of normal morphology would potentially lead to a lower ratio of cases to controls, with a concomitant reduction in power. Future studies comparing the different definitions of 'normal morphology' should take this into account before like-for-like comparisons are made.

The strengths and limitations of our study have already been described (Cherry et al., 2008; Povey et al., 2012). Limitations include the fact that: (i) men who participated may not be representative of all men in couples with fertility problems; (ii) low-income couples who knew they could not afford any subsequent fertility treatment may have declined to attend for the free baseline investigations; (iii) men who refused to participate may have done so because they had a lifestyle they did not want to declare or (iv) that men may have underestimated their exposure to certain lifestyle factors as they were aware that as a couple they were being investigated for infertility. With regard to the latter (if present) we suggest that this would more likely impact on the power of the study than introduce bias since any underestimation would be unlikely to differ between cases and controls, as case status was unknown to the participant before the questionnaires were completed.

The fact that data about men's lifestyle were collected before they knew the results of their semen analysis is a major strength of our study design and serves to minimize the possibility of bias. Therefore, although we must accept that some degree of misclassification exists, we can reasonably conclude that our negative findings do not result from differential misclassification of self-reported exposures between cases and controls. Our previous reports using the same data set (e.g. Cherry et al., 2008; Povey et al., 2012; Iszatt et al., 2013) have given plausible biological results for low motile sperm concentration and we have no reason to suspect that by using poor morphology as the case definition this should be any different. A second strength arises from the central assessment of sperm morphology in a single laboratory, thereby removing the

possibility of measurement errors in each of the collaboration centres (Pacey, 2006; 2010) that are particularly pertinent to measurement of sperm morphology (Riddell *et al.*, 2005). To improve the consistency of sperm morphology measurements we also chose to use a computerized system, which has the advantage of providing greater objectivity, precision and reproducibility than examining sperm morphology smears by eye (WHO 2010). A final advantage is the fact that this large, multi-centre, study has the statistical power to uncover relatively small effects, at least for a common exposure. Given the observed numbers of cases and controls, the study had 80% power to detect an OR of 1.43 or higher for smoking; therefore, we are confident of our conclusion that, for example, cigarette smoking in the 3 months before the semen sample was collected has little detrimental effect on sperm morphology. For alcohol consumption exceeding 50 units a week (i.e. $\geq$456 units in 3 months), there was 80% power to detect an OR of 1.81 or higher, so we can confidently exclude larger effects than this.

In conclusion, this study has identified few modifiable factors associated with poor sperm morphology, with the only practical advice to men attempting conception being to limit exposure to cannabis if they are regular users. Whether alterations to sexual abstinence or season of sample production (i.e. avoiding samples for assisted conception produced in summer) have any practical advantage, remain to be established. Overall, we would argue that the results of this study, in combination with our papers that investigated the effect of lifestyle (Povey *et al.*, 2012) and occupation (Cherry et. al., 2008) on poor motile sperm concentrations, suggest that men can make relatively few lifestyle changes to improve semen quality either to enhance natural conception or improve their chances in assisted conception. The strength of inference would be greater if there were data from an intervention study of behavioural change—and we cannot exclude the possibility that such a study would find beneficial effects—but the balance of present evidence does not suggest that the benefit would be large.

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Authors' roles

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. Data management was done by J.-A.C., H.B., N.C. and H.M.. A.A.P., A.C.P., R.M. and N.C. drafted the manuscript and 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. A.A.P. and N.C. are guarantors.

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Conflict of interest

None declared.

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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.