ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

ORIGINAL ARTICLE

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Keywords:
BMI, dose–response association, education, hospitalization, lifestyle, occupation, semen quality, smoking, sperm concentration, total sperm count

Received: 15-Sep-2017
Revised: 26-Jan-2018
Accepted: 28-Jan-2018

doi: 10.1111/andr.12477

Semen quality associated with subsequent hospitalizations – Can the effect be explained by socio-economic status and lifestyle factors?

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SUMMARY

Semen quality is suggested to be a universal biomarker for future health. Previous studies have mostly been registry based excluding the possibility to address the importance of lifestyle, fertility status, health and socio-economic status. We aimed to investigate whether the association between semen quality and subsequent risk of hospitalization could be explained by differences in occupation, education, fertility, cryptorchidism, BMI or smoking; 1423 men with first semen sample at Fertility Clinic, Frederiksberg Hospital, Denmark, from 1977 to 2010 responded to a questionnaire in 2012 about current health, lifestyle, educational level and occupation. They were followed in the Danish National Patient Registry to first-time hospitalizations using ICD-8 and ICD-10 classification. Data were analysed by Cox proportional hazard regression models to adjust for the possible confounding factors. We found a significant higher risk of being hospitalized with decreasing sperm concentrations (0–15 mill/mL: HR1.78, 95% CI:1.51–2.09; 16–50 mill/mL: HR 1.37 95% CI: 1.17–1.60; 51–100 mill/mL: HR1.25 95% CI: 1.07–1.45). Same significant association of being hospitalized with decreasing total sperm counts was seen. The dose–response increase in risk in hospitalization with decreasing sperm concentration and total sperm count remained constant after further individual adjustment for occupation, marital status, fertility, cryptorchidism, BMI or smoking. The association between semen quality and subsequent morbidity was not explained by differences in lifestyle, behavioural or fertility status. We were unable to adjust for all possible confounders simultaneously due to limited sample size, and reverse causation is a possible explanation as information about education and lifestyle was obtained after semen analysis and hospitalizations occurred and may have changed as consequence of both. Semen quality may be a universal biomarker for future health not explained by lifestyle and socio-economic status, but this needs to be addressed further in future studies.

INTRODUCTION

In 1992, the first review was published suggesting a decline in semen quality during a 50-year period (Carlsen *et al.*, 1992). This was recently followed up with a meta-analysis covering the last 25 years, which suggested that the decline has continued among Western men (Levine *et al.*, 2017) underlining the actuality and public health importance of the problem. In addition, male factor infertility contributes to more than half of all cases of global involuntary childlessness (Inhorn & Patrizio, 2015).

The evidence is emerging that semen quality is not only a marker for fertility but also a universal biomarker of health as several studies have found associations between semen quality and subsequent morbidity and mortality (Eisenberg *et al.*, 2016; Jensen *et al.*, 2009). A large Danish cohort study with 40 years of follow-

up showed higher mortality among men with poor semen quality compared to men with good semen quality. This was detected in both, among men who subsequently fathered a child and men who remained childless, even though childless men had a shorter life expectancy than men who fathered a child (Jensen *et al.*, 2009). Another newly published US study detected higher morbidity in a large group of men seeking fertility care (Eisenberg *et al.*, 2016). Our recent study demonstrated a higher risk of hospitalization due to all causes and particularly cardiovascular diseases and diabetes (Latif *et al.*, 2017) among men with low sperm concentration compared to men with high sperm concentration. However, the results were based on registry data with limited individual information and no information on smoking, BMI, fertility and socio-economic status (SES). Importantly, smoking and obesity are known to adversely influence not only semen parameters, but also general health and life expectancy (Jensen *et al.*, 2004; Ramlau-Hansen *et al.*, 2007; Li *et al.*, 2011; Jurewicz *et al.*, 2014). In addition, several studies have reported associations between SES and morbidity and mortality with up to 10 years of shorter life expectancy among men from lower SES compared to higher SES (Marmot, 2005; Clark *et al.*, 2009; Deans *et al.*, 2009; Baadsgaard & Broennum-Hansen, 2012). It is therefore difficult to rule out the possibility that our findings of higher morbidity (e.g. hospitalizations) among men with low sperm concentration could be explained by differences in lifestyle, BMI and socio-economic status (SES) associated with both semen quality and morbidity.

In our previously register-based study among 4501 Danish men evaluated for infertility (Latif *et al.*, 2017), 1854 of these responded to a questionnaire about current health, lifestyle and socio-economic status. We therefore used the questionnaire information to investigate whether the association between semen quality and subsequent risk of hospitalization is affected by differences in SES, BMI, smoking and fertility status.

MATERIAL AND METHODS

Study population

The cohort from the Fertility Clinic at Frederiksberg Hospital, Copenhagen, Denmark, has previously been described (Latif *et al.*, 2017). A total of 4501 men who had previously been evaluated for infertility in Frederiksberg Hospital from 1996 to 2010 were in 2012 followed up in registers for hospitalizations (Latif *et al.*, 2017). They were also invited to participate in a study regarding the impact of semen quality on future health and to fill in a self-administrated questionnaire (Joergensen *et al.*, 2009). A total of 1854 men responded to a questionnaire from which 240 men were excluded due to a semen analysis performed before 1977, where the National Patient Registry (NPR) was introduced, as were 191 men who were hospitalized prior to semen analysis. Finally, 1423 men who delivered a semen sample at Frederiksberg Hospital from 1977 to 2010 and responded to a questionnaire were included in this study.

Records of hospitalization were obtained by linking to the NPR using the unique personal identification number given to all Danish citizens from 1968 and to all newborns and immigrants thereafter (Schmidt *et al.*, 2014). The NPR was established in 1977 and hold information on all hospitalizations and inpatient contacts with hospitals in Denmark (Schmidt *et al.*, 2015). We recorded all first-time hospitalizations and used ICD-8 and ICD-10 as our main diagnostic tools. All inpatient admissions were recorded from 1977 until 1st of August 2015 or death.

Questionnaire

All men were mailed a questionnaire in Danish at their home address and asked to return these in a pre-payed envelope. They provided information about current height and weight (from which BMI was calculated); previous cryptorchidism; marital status (married, unmarried, separated, registered partnership or widower); and whether they had any biological or adopted children and if so whether they were conceived before or after the semen samples were delivered. They were asked whether they had ever smoked or whether they were ex-smokers, non-smokers or current smokers. They provided information about start and frequency of smoking, average number of cigarettes per week, how many years they had smoked, also if they smoked other types of tobacco, for example pipes, cigarillos, cigars, pipe tobacco or hookah. Current smokers were defined as daily smokers or 'party smokers' who smoked at least once a week. Ex-smokers were defined as smokers who had stopped smoking within the last 6 months and non-smokers as men who never smoked. The questionnaire also contained questions about their highest level of education (secondary school, A-levels/college, foundation degree, bachelor degree or master degree, vocational training or other type of educations). Men were also queried whether they were currently unemployed, skilled or unskilled worker, self-employed, white-collar worker or retired. If they had retired, they were asked about their previous occupation.

Semen analysis

We used the results of the first semen sample for each man delivered for analysis due to couple infertility. Prior to delivery of these samples, the men were advised to keep an ejaculation abstinence period of three to four days. The actual abstinence periods were recorded when the samples were delivered to the laboratory. The semen samples were produced at home and brought to the laboratory protected from extreme temperatures within one hour after ejaculations. The samples were kept at room temperature in the laboratory during the analysis. A dedicated laboratory technician, who worked in the laboratory for a period of 40 years, performed the analysis during the whole period. Semen volume was assessed by aspiration and sperm concentration subsequently using improved Neubauer haemocytometers. Total sperm count was calculated as semen volume $\times$ sperm concentration.

The cut-off values and definitions for semen analysis were determined according to latest recommendations of World Health Organization (WHO) lower reference values as semen volume <1.5 mL, sperm concentration <15 mill/mL and total sperm count as <39 mill were used (WHO, 2010; Cooper *et al.*, 2012).

From 1977 to 2010, the laboratory worked in close collaboration with other Nordic laboratories and followed the guidelines from quality control groups under Nordic Association for Andrology (NAFA), who facilitated the establishment of common standardized methods and materials by recommended guidelines from WHO in andrology laboratories in the Nordic countries. During the whole period, the laboratory met the criteria for external quality controls and no adjustments were needed.

Statistical analysis

Initially mean, standard deviation, median and range of semen volume, sperm concentration and total sperm count were calculated. All participants were grouped into high (A-level/college and above) and low education (under A-level/college) to test distribution of lifestyle and behavioural factors between groups by Pearson's chi-square test.

We then calculated time from semen analysis to the first hospitalization using the Kaplan–Meier survival estimation and diagrams. To examine for possible confounders in the association between sperm parameters and risk of hospitalization, we used both Cox regression analysis and logistic regression. For the logistic regression analysis, semen volume, sperm concentration and total sperm count were dichotomized into <1.5 mL and above, <15 mill/mL and 15 mill/mL or more and <39 mill and above (Cooper *et al.*, 2012) as outcome and regressed against the possible confounders with adjustment of age at the time of semen analysis and year of birth. Odds ratio (OR) and hazard ratios (and 95% confidence intervals) were calculated for each possible confounder to examine the individual effect on sperm concentration, total sperm count and hospitalizations. Sperm concentration and total sperm count were categorized based on WHO cut-off points of, respectively, 15 mill/mL and 39 mill (WHO, 2010). To examine the association between possible confounders on the risk of hospitalization among men with different sperm concentrations (0–15, 16–50, 51–100 and >100 mill/mL) and total sperm counts (0–39 and 40–120 and >120 mill), a Cox proportional hazard regression model was used and the hazard ratio (HR) for hospitalization was adjusted for age at semen analysis and year of birth (to avoid birth cohort effect). We further adjusted the HR for age at semen analysis, year of birth and one of the following factors; occupation, education, fertility, cryptorchidism, BMI and smoking one at a time to test whether these adjustments changed the HR. We did not adjust for all possible confounders simultaneously due to the restrictions in sample size. In all analyses, the Cox proportional hazards assumption was fulfilled after categorization of variables. We used the score process to test the assumption (Lin *et al.*, 1993), and analyses were performed using the ASSESS statement in PROC PHREG (SAS, version 9.4; SAS Institute, Inc., Cary, NC, USA).

RESULTS

A total of 1423 included men were followed until date of first hospitalization or end of study period (1st of August 2015) with a mean time to first hospitalization of 5.9 years (standard error, 0.17) and maximal of 36.2 years. Average age at time of semen analysis was 33.3 years (range 18–59 years) with a mean sperm concentration of 73 mill/mL and median of 44 mill/mL (range 0–443), mean total sperm count of 228 mill and a median of 171 mill (range 0–1580). The average age at the time of questionnaire response was 51.9 years (range 26–84 years). We found no differences in semen characteristics or age between men who responded to the questionnaire and non-responders (Table S1).

We compared hospitalizations among the men included in the study (N = 1423) with the total population included in the previous study (N = 4501) (Latif *et al.*, 2017); we therefore compared hospitalizations among 3078 men (4501–1423) followed up in NPR with no questionnaire data with 1423 men with both NPR and questionnaire follow-up. The latter group was less hospitalized than non-responders representing a healthier population (Figure S1).

Most men were well educated and had a master degree and were white-collar workers or self-employees. We had no non-smokers, and we therefore grouped the participants into current smokers and ex-smokers. Men with low education were more often overweight, current smokers and reported more cases of cryptorchidism compared to the high-educated men. The high-educated men were primary white-collar workers, married and reported no biological children compared to the low educated (data not shown).

Semen volume in the initial descriptive analysis was not associated with hospitalizations, and we only included sperm concentration and total sperm count in further analyses.

The Kaplan–Meier plots showed increased risk of hospitalizations among men with sperm concentration below 15 mill/mL compared to men with a concentration above 15 mill/mL and with a total sperm count below 39 mill compared to above 39 mill, both among low- and high-educated men, smokers and non-smokers and men with normal or high BMI (Fig. 1).

Occupation, education, marital status, smoking or BMI were not associated with low sperm concentration below 15 mill/mL and low sperm count below 39 mill (Table 1). The odds ratios (OR) correspond to sperm concentration below 15 mill/mL and sperm count below 39 mill for each characteristic. OR for having a sperm concentration below 15 mill/mL was 3.03 (95% CI: 2.07–4.42), and OR for total sperm count below 39 mill was 3.26 (2.26–4.74) among men with cryptorchidism and 2.63 (95% CI: 1.95–3.55) among men with no biological children, also reflected in OR for having a total sperm count below 39 mill, respectively, 3.26 (2.25–4.74) and 2.60 (1.93–3.50) as shown in Table 1.

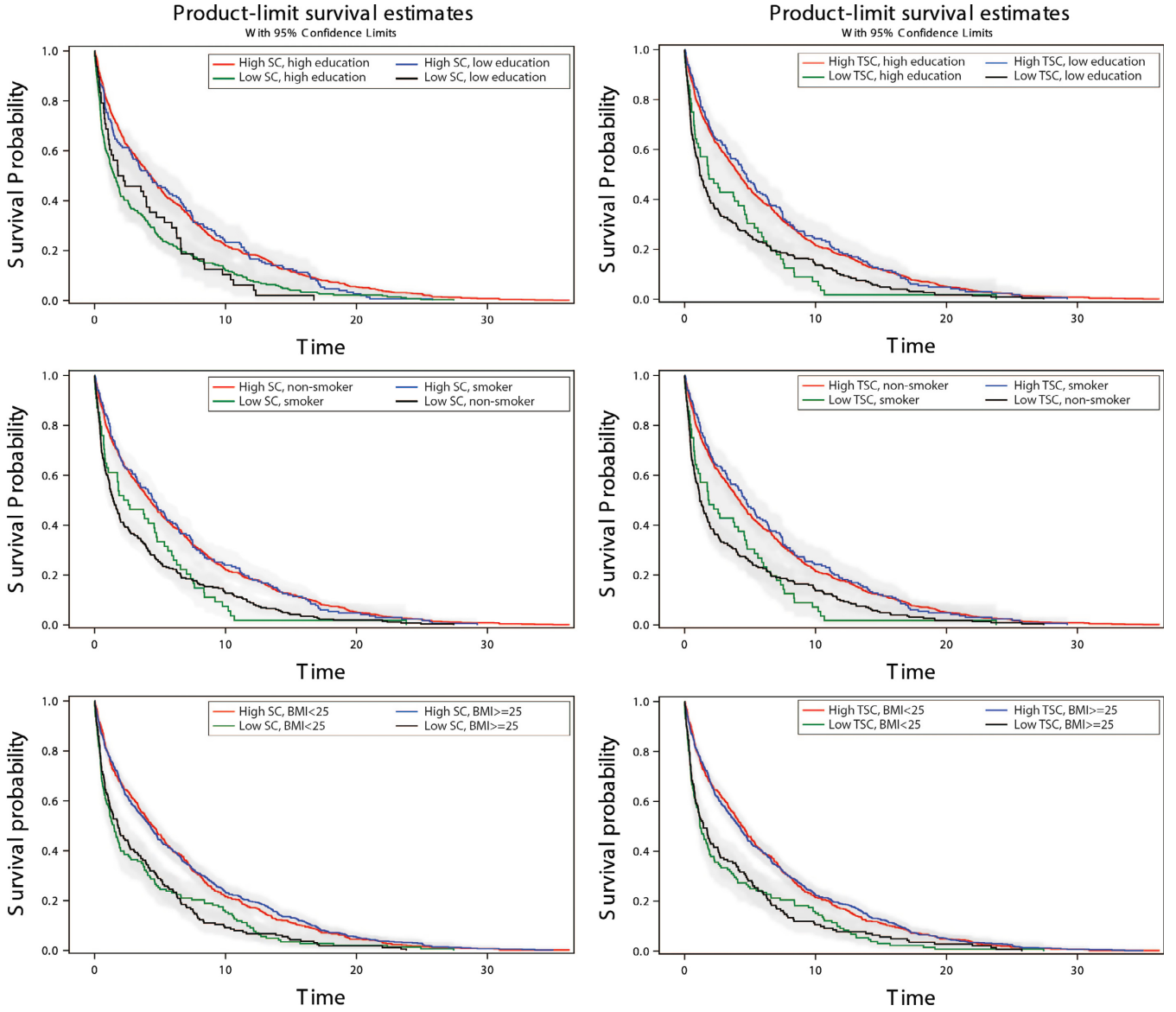
Sperm concentration and total sperm count were associated with a HR of hospitalization in a dose–response pattern after adjustments for age at semen analysis and year of birth (Table 2, Fig. 1). Occupation, education, fertility status, marital status, smoking and BMI were not associated with risk of hospitalization (Table 2).

A sperm concentration 0–15, 16–50 and 51–100 mill/mL and total sperm count <39 and 40–120 mill were also associated with a HR of hospitalization in a dose–response pattern compared to a concentration >100 mill/mL and total sperm count >120 mill after adjustment for age, year of birth and education (Table 3). Men with a sperm concentration 0–15, 16–50 and 51–100 mill/mL had, respectively, 78%, 37% and 25% increased risk of being hospitalized compared to men with a sperm concentration above 100 mill/mL (Table 3). The same increased risk of being hospitalized with decreasing total sperm count was seen (Table 3). The dose–response increase in risk in hospitalization with decreasing sperm concentrations and total sperm counts remained remarkably constant after further individual adjustment for occupation, marital status, fertility, cryptorchidism, BMI or smoking (Table 3).

DISCUSSION

In this retrospective cohort study, among 1423 men referred for semen analysis at a fertility clinic from 1977 to 2010 and followed up for 36 years; we detected a dose–response association between sperm concentration and total sperm count and risk of hospitalization. Men with a sperm concentration 0–15, 16–50 and 51–100 mill/mL and total sperm count below 39 mill had

Figure 1 Probability of 'survival' from first-time hospitalization among men with a sperm concentration 0–15 mill/mL [low sperm concentration (SC)] compared to men with a sperm concentration >15 mill/mL [high sperm concentration (SC)], total sperm count 0–39 mill [low total sperm count (TSC)] compared to men above 39 mill [high total sperm count (TSC)] with either high or low education (high = A-levels/College and more; Low = under A-levels/College) smoking or not smoking and high (>25 kg/m^2) or low (<25 kg/m^2) BMI among 1423 infertile men investigated due to couple infertility from 1977 to 2010. Shaded areas are 95% confidence intervals.



increased risk of being hospitalized compared to men with a sperm concentration above 100 mill/mL and a total sperm count above 120 mill. Adjustment for education, occupation, fertility, marital status, BMI or smoking did not change the estimates. However, we were unable to adjust for all possible confounders simultaneously due to limited sample size. In addition, reverse causation is a possible explanation as information about these factors was obtained after semen analysis and hospitalizations occurred and therefore may have changed as consequence of both. Semen quality may therefore represent a universal biomarker for subsequent morbidity.

Our long-term follow-up study of 4501 Danish men evaluated for infertility demonstrated that men with the poorest semen quality had their first hospitalization seven years earlier than men with the best semen quality (Latif *et al.*, 2017). An American study compared 13,027 men diagnosed with infertility and followed for 9 years to men who were only tested for infertility and found a 48% and a 30% increase in risk of for developing ischaemic heart disease and diabetes between the two groups; however, no semen analysis was performed in this study (Eisenberg *et al.*, 2016). A large Danish cohort study of 43,277 men found a 45% lower mortality among men with a sperm concentration above 40 mill/mL compared to men with sperm concentration below this level, both among men with and without biological children, even though men with children had a lower mortality than childless men (standard mortality ratio: 1.89, 95% CI: 1.67, 2.14) (Jensen *et al.*, 2009). None of these studies adjusted for social class, BMI or lifestyle factors.

Overweight and smoking are well-known risk factors for increased incidence of multiple comorbidities including type II diabetes, cancer and cardiovascular diseases (Guh *et al.*, 2009; Li *et al.*, 2011; Jurewicz *et al.*, 2014). A prospective US study

Table 1 Adjusted odds ratio (OR) and 95% confidence interval (95% CI) for sperm concentration below 15 mill/mL and total sperm count below 39 mill among 1423 men investigated due to couple infertility from 1966 to 2010 according to different behavioural, lifestyle factors and fertility status

Characteristics	*N*	Sperm concentration <15 mill/mL OR 95% CI*	Total sperm count <39 mill OR 95% CI*
Occupational status			
Unemployed	26	0.75 (0.25–2.28)	0.70 (0.23–2.13)
Unskilled worker	43	0.91 (0.41–2.03)	0.94 (0.42–2.08)
Skilled worker	101	0.77 (0.44–1.35)	0.99 (0.58–1.68)
Retired	114	2.06 (1.09–3.91)	2.24 (1.20–4.16)
Self-employed (liberal professions, agriculture, other)	256	0.82 (0.57–1.19)	1.07 (0.75–1.52)
White-collar worker	816	Reference	Reference
Education level			
Other education	19	2.68 (0.86–8.32)	1.76 (0.54–5.72)
A-levels/College	123	0.46 (0.20–1.05)	0.42 (0.18–0.96)
Vocational training	123	0.69 (0.32–1.52)	1.07 (0.50–2.28)
Foundation degree	149	0.90 (0.43–1.90)	1.02 (0.48–2.16)
Bachelor's degree	345	0.70 (0.35–1.13)	0.72 (0.36–1.44)
Master's degree	589	0.58 (0.29–1.41)	0.65 (0.33–1.28)
Secondary school	57	Reference	Reference
Children			
No biological children	1082	2.63 (1.95–3.55)	2.60 (1.93–3.50)
Biological children	341	Reference	Reference
No biological children before semen analysis	1141	1.18 (0.81–1.73)	1.17 (0.80–1.70)
Biological children before semen analysis	246	Reference	Reference
Adopted children no	1289	0.99 (0.59–1.66)	0.97 (0.58–1.61)
Adopted children yes	120	Reference	Reference
Marital status			
Married	1014	0.85 (0.61–1.20)	0.71 (0.51–0.99)
Separated	81	0.90 (0.47–1.71)	0.90 (0.48–1.67)
Registered partnership	31	0.36 (0.10–1.24)	0.32 (0.09–1.12)
Widower	8	1.38 (0.26–7.46)	2.22 (0.49–10.20)
Unmarried	252	Reference	Reference
Smoking			
Ex-smokers	621	0.95 (0.72–1.25)	0.82 (0.62–1.09)
Current smokers	793	Reference	Reference
BMI kg/m^2			
Normal weight (19–25)	649	0.91 (0.69–1.19)	1.03 (0.79–1.35)
Overweight (>25)	723	Reference	Reference
Genital birth defect			
Cryptorchidism	166	3.03 (2.07–4.42)	3.26 (2.25–4.74)
Cryptorchidism no	870	Reference	Reference

*, adjusted.

investigated the impact of SES and health behaviours on mortality and found a HR of 2.77 among participants with low SES compared to high SES after a follow-up period of 7.5 years (Lantz *et al.*, 1998). Obesity and smoking have also been associated with poor semen quality (Jensen *et al.*, 2004; Ramlau-Hansen *et al.*, 2007; Jurewicz *et al.*, 2014; Andersen *et al.*, 2015). A higher risk of abnormal sperm counts in overweight and obese men compared to men of normal weight has been found (Bonde *et al.*, 1998; Jensen *et al.*, 2002; Sermondade *et al.*, 2013). A newly published meta-analysis showed that exposure to cigarette smoking was associated with reduced semen quality, and when stratified in subgroups, the effect size was higher in the group of infertile men compared to men in general (Sharma *et al.*, 2016). As education, smoking and BMI are associated with both semen quality and morbidity, these factors may powerfully confound the association between semen quality and subsequent morbidity. However, this was not the case in our study, as our hazard ratios remained constant after adjustment for all SES and lifestyle factors suggesting that semen quality may be an independent biomarker for morbidity. However, information on lifestyle and SES was obtained after semen analyses and hospitalizations occurred and the men may have changed their lifestyle or occupation as consequence of poor semen quality or hospitalization.

The aetiology for the association between semen quality and morbidity remains uncertain. However, several plausible hypotheses exist. Approximately 15% of the genome is involved in reproduction and (Matzuk & Lamb, 2008) could mediate the link between semen quality and subsequent morbidity given the redundancy of function of genes across several organ systems. Next, semen quality is also associated with circulating testosterone levels with infertile men having lower testosterone than fathers (Andersson *et al.*, 2003; Jensen *et al.*, 2004; Meeker *et al.*, 2007). Moreover, testosterone deficiency predicts not only later morbidity but also mortality as the association between low testosterone levels and risk of CVD has been found in several studies (Stellato *et al.*, 2000; Laaksonen *et al.*, 2004; Araujo *et al.*, 2011; Oskui *et al.*, 2013). In addition, abnormal genital development could lead to poor semen quality and associations with other types of urogenital malfunctions (Matzuk & Lamb, 2008). The increase in hypospadias, cryptorchidism and testicular cancer has coincided with decline in semen quality (Carlsen *et al.*, 1992; Bonde *et al.*, 1998; Moller & Skakkebaek, 1999; Toppari *et al.*, 2001). These conditions are suggested to be of mutual risk factors for each other, which are developed in the embryonic stage and suggested to be different manifestations of an underlying syndrome – called testicular dysgenesis syndrome (Skakkebaek, 2014). Other studies have demonstrated that in utero

Table 2 Adjusted hazard ratio and 95% confidence interval (95% CI) for all-cause hospitalization among 1423 men investigated due to couple infertility from 1966 to 2010 according to different behavioural, lifestyle factors and fertility status

Characteristics*	*N*	Hospitalization all causes HR 95%CI*
Semen volume mL		
0–1.5	143	1.03 (0.87–1.23)
>1.5	1275	Reference
Sperm concentration mill/mL		
<15	290	1.79 (1.53–2.11)
16–50	373	1.36 (1.17–1.58)
51–100	375	1.24 (1.07–1.44)
>100	361	Reference
Total sperm count mill		
0–39	282	1.71 (1.49–1.96)
40–120	284	1.21 (1.05–1.38)
>120	857	Reference
Occupational status		
Unemployed	26	1.28 (0.86–1.90)
Unskilled worker	43	1.12 (0.82–1.52)
Skilled worker	101	1.18 (0.96–1.46)
Retired	114	1.23 (0.94–1.61)
Self-employed (liberal professions, agriculture, other)	256	1.10 (0.96–1.27)
White-collar worker	816	Reference
Education level		
Other education	19	0.77 (0.45–1.29)
A-levels/College	123	0.72 (0.52–0.99)
Vocational training	123	0.95 (0.69–1.31)
Foundation degree	149	1.02 (0.75–1.39)
Bachelor's degree	345	0.83 (0.63–1.11)
Master's degree	589	0.75 (0.57–0.99)
Secondary school	57	Reference
Children		
No biological children	1082	1.11 (0.98–1.26)
Biological children	341	Reference
No biological children before semen analysis	1141	0.96 (0.83–1.11)
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Adopted children		
Adopted children no	1289	1.06 (0.87–1.29)
Adopted children yes	120	Reference
Marital status		
Married	1014	0.96 (0.83–1.11)
Separated	81	1.12 (0.87–1.44)
Registered partnership	31	1.47 (1.01–2.15)
Widower	8	1.64 (0.78–3.42)
Unmarried	252	Reference
Smoking		
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Current smokers	793	Reference
BMI kg/m^2		
Normal weight (19–25)	649	0.91 (0.81–1.01)
Overweight (>25)	723	Reference
Genital birth defect		
Cryptorchidism	166	1.26 (1.06–1.49)
No cryptorchidism	870	Reference

*, adjusted.

exposure to environmental or maternal toxicants/stressors could lead to poor health but also poor reproduction in men (Godfrey & Barker, 2000; Virtanen *et al.*, 2005; Phillips & Foster, 2008; Phillips & Tanphaichitr, 2008). The same underlying exposures during vulnerable stages of testis development may therefore lead not only to poorer semen quality but also to increased morbidity, supported by our findings of an association between poor semen quality and subsequent hospitalization risk, which was not affected by lifestyle, BMI or SES. This suggests that infertile men with low sperm counts should be followed up with regularly health examinations as they are at increased risk for the disease.

Strengths and limitations

The major strength of our study is the long-term follow-up and the combination of a comprehensive follow-up in a population-based registry of high quality combined with questionnaire-based information education, occupation, fertility or marital status, BMI and smoking. The responders were less hospitalized than non-responders and could represent a healthier population who usually are more likely to respond questionnaires.

All participants were referred for infertility assessment and therefore do not represent the general population. Couples seeking fertility assessment are more often married, older and with a higher educational level than the general population (Hotaling *et al.*, 2012) consisting with our study population as they were married and well educated. We compared hospitalization between groups so whether the men represented the general population is of less importance.

In addition, the response rate to questionnaire was low (36.5%) representing a possible selection bias. However, the age and semen quality were similar among responders and non-responders to questionnaires making differential selection less likely. A total of 74 men (1.4%) from the original cohort died before the studies were conducted. These men may be more prone to diseases than the surviving men included in our study and probably had an unhealthier lifestyle than the participants. It could also be a manifestation of the impaired general health associated with the low sperm counts. Our study population may therefore be healthier than the general population, thereby underestimating the association between poor semen quality and morbidity. The fact that the men who died had a poorer semen quality than the men included confirms this. However, we believe that the probable impact on our results is limited due to the low number of deaths. Furthermore, a total of 218 men (4.1%) with poorer semen quality emigrated before our study and inclusion of these including the men who died would have strengthened our findings.

Information obtained about lifestyle factors and SES was self-reported based on current status not at the appropriate time of semen analysis and after smoking reverse causation a possible explanation of our findings. Men with poor semen quality or men who have been hospitalized are probably more likely to have changed their lifestyle causing not only differential misclassification. If these men are more likely to have stopped smoking than the others, they will be misclassified as non-exposed, thereby underestimating the effect of smoking. Similarly, overweight men with poor semen quality or who have been hospitalized may be more likely to reduce weight, thereby underestimating the effects of BMI. However, BMI may also be on the causal pathway between semen quality and hospitalizations, if semen quality is a general biomarker for health, and men with poor semen quality therefore more often become overweight and therefore are hospitalized. All included men had been smokers which was not unusual at the time of inclusion as in the 1970s 70% of Danish men were smokers (Danish National Health Board, 2007), thereby making it impossible to study the effect of smoking and the potential alterations this could have had on our results. As all men had been smokers, it may explain why we found no association between smoking and hospitalizations.

Table 3 Hazard ratio (HR) and 95% confidence interval (95% CI) for hospitalization among men with different sperm concentration and total sperm count after adjustment of age, year of birth, education and one of behavioural, lifestyle factor or fertility status

Models	Sperm concentration mill/mL				Total sperm count mill		
	0–15 HR 95%CI	16–50 HR 95%CI	51–100 HR 95%CI	>100	0–39 HR 95%CI	40–120 HR 95%CI	>120
Univariate							
Age*	2.18 (1.86–2.56)	1.87 (1.61–2.17)	1.51 (1.31–1.75)	Reference	1.72 (1.50–1.97)	1.34 (1.17–1.54)	Reference
Year of birth	1.94 (1.66–2.28)	1.47 (1.27–1.71)	1.32 (1.14–1.54)	Reference	1.80 (1.57–2.06)	1.25 (1.09–1.43)	Reference
Age* and year of birth	1.79 (1.53–2.11)	1.36 (1.17–1.58)	1.24 (1.07–1.44)	Reference	1.71 (1.49–1.97)	1.21 (1.05–1.38)	Reference
Age*, year of birth and Education	1.78 (1.51–2.09)	1.37 (1.17–1.60)	1.25 (1.07–1.45)	Reference	1.70 (1.48–1.95)	1.20 (1.04–1.37)	Reference
Adjusted for age, year of birth, education and one of the following confounders							
Occupational status	1.80 (1.53–2.12)	1.35 (1.16–1.57)	1.24 (1.07–1.44)	Reference	1.72 (1.49–1.98)	1.19 (1.04–1.37)	Reference
Marital status	1.80 (1.53–2.11)	1.35 (1.16–1.58)	1.24 (1.07–1.44)	Reference	1.70 (1.48–1.96)	1.20 (1.05–1.38)	Reference
No biological children	1.78 (1.52–2.10)	1.36 (1.16–1.58)	1.24 (1.07–1.44)	Reference	1.70 (1.48–1.96)	1.21 (1.05–1.38)	Reference
Biological children before semen analysis	1.80 (1.53–2.11)	1.36 (1.17–1.58)	1.24 (1.07–1.44)	Reference	1.72 (1.50–1.97)	1.21 (1.05–1.39)	Reference
Adopted children	1.80 (1.53–2.11)	1.36 (1.17–1.58)	1.24 (1.07–1.58)	Reference	1.72 (1.50–1.98)	1.21 (1.05–1.38)	Reference
Cryptorchidism	1.77 (1.50–2.08)	1.35 (1.16–1.58)	1.24 (1.07–1.44)	Reference	1.68 (1.46–1.94)	1.20 (1.04–1.37)	Reference
BMI kg/m^2	1.81 (1.53–2.12)	1.37 (1.17–1.59)	1.24 (1.07–1.44)	Reference	1.72 (1.50–1.97)	1.20 (1.05–1.38)	Reference
Smoking	1.79 (1.53–2.10)	1.35 (1.16–1.58)	1.23 (1.06–1.43)	Reference	1.72 (1.50–1.98)	1.21 (1.05–1.38)	Reference

*, adjusted.

Such analysis would be of high interest to pursue in future studies.

We adjusted for relevant confounders, for example education, occupation, fertility, marital status, BMI or smoking, but did not take into account other important confounding factors like exercise, diet and other diseases. The sample size restricted our possibility to adjust for all confounders simultaneously.

CONCLUSIONS

We found a dose–response association between sperm concentration, total sperm count and subsequent risk of hospitalization. This association persisted after individual adjustment for education, occupation, fertility, marital status, BMI or smoking. We did, however, not have a large enough sample size to adjust for all factors simultaneously. In addition, reverse causation is a possible explanation to our findings as information on lifestyle and socio-economic factors was obtained after semen quality and hospitalizations. Semen quality may represent a universal biomarker for morbidity, but further studies with information on relevant factors at the right exposure window are needed.

CONFLICT OF INTEREST

The authors report no conflict of interests. The authors alone are responsible for writing the study.

FUNDING

This work was supported by Department of Environmental Medicine, Faculty of Health Sciences, University of Southern Denmark and the Coordinating Research Centre, Bispebjerg & Frederiksberg Hospitals.

AUTHORS' CONTRIBUTIONS

Tabassam Latif is the primary author together with Tina Kold Jensen and has written the manuscript. Rune Lindahl-Jacobsen and Tina Kold Jensen contributed to the hypothesis and supported by Niels Joergensen, Jesper Mehlsen and Sven Olaf Skouby for the idea of the study and study design. Stine Agergaard Holmboe, Kirsten Pors and Louise Brinth helped with the data collection, and statistical analysis was highly supported by Rune Lindahl-Jacobsen. Rune Lindahl-Jacobsen, Tina Kold Jensen, Michael Eisenberg and Niels Joergensen revised critically for important intellectual content and contributed to the data interpretation. The article is coedited and finally approved by all authors.

ETHIC APPROVAL

The Ethical Committee for the Capital Region of Denmark approved the study in June 2011.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Annual survival probability without hospitalizations from 1977 to 2010 among 3078 men investigated due to couple infertility and followed up in National Patient Register with no questionnaire data (red line) and among 1423 men with both register and questionnaire follow-up (blue line).

Table S1 Semen characteristics of men investigated due to couple infertility from 1966 to 2010 stratified according to whether or not they responded to questionnaire in 2012.

