American Journal of Epidemiology

Vol. 186, No. 8
DOI: 10.1093/aje/kwx067
Advance Access publication:
May 11, 2017

Original Contribution

Semen Quality as a Predictor of Subsequent Morbidity: A Danish Cohort Study of 4,712 Men With Long-Term Follow-up

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Initially submitted August 4, 2016; accepted for publication December 6, 2016.

Semen quality has been suggested to be a biological marker of long-term morbidity and mortality; however, few studies have been conducted on this subject. We identified 5,370 men seen for infertility at Frederiksberg Hospital, Denmark, during 1977–2010, and 4,712 of these men were followed in the Danish National Patient Registry until first hospitalization, death, or the end of the study. We classified patients according to hospitalizations and the presence of cardiovascular disease, diabetes, testicular cancer, or prostate cancer. We found a clear association between sperm concentration below 15 million/mL and all-cause hospitalizations (hazard ratio = 1.5, 95% confidence interval: 1.4, 1.6) and cardiovascular disease (hazard ratio = 1.4, 95% confidence interval: 1.2, 1.6), compared with men with a concentration above 40 million/mL. The probabilities for hospitalizations were also higher with a low total sperm count and low motility. Men with a sperm concentration of 195–200 million/mL were, on average, hospitalized for the first time 7 years later than were men with a sperm concentration of 0–5 million/mL. Semen quality was associated with long-term morbidity, and a significantly higher risk of hospitalization was found, in particular for cardiovascular diseases and diabetes mellitus. Our study supports the suggestion that semen quality is a strong biomarker of general health.

cardiovascular disease; diabetes; hospitalization; prostate cancer; sperm concentration; testicular cancer; total sperm count

Abbreviations: CI, confidence interval; ICD, *International Classification of Diseases*.

Approximately 15% of all couples experience infertility, and male factor issues are responsible for 50% of fertility problems (1–3). Young Scandinavian men have a median sperm concentration of 44 million/mL (4), close to 40 million/mL, a level below which the probability of conception has been found to be reduced (2). Impaired semen quality has been linked to a higher risk of testicular cancer in men in the years following an infertility evaluation, among both Danish and US men (5–10), and some studies have suggested a link to prostate cancer, although this has not been confirmed by all studies (11, 12). Semen quality has been linked to mortality (13, 14) and, recently, also to morbidity from a wide range of diseases (15), suggesting that semen quality could be a universal health marker.

The link between semen quality and health later in life could be due to genetic, hormonal, lifestyle, or in utero factors. Because approximately 15% of the male human genome is involved in reproduction, it is conceivable that other health ailments would be linked to defects in fertility (16). Low testosterone level has also been associated both with low semen quality and with subsequent morbidity and mortality (17–19). However, the association between semen quality and health later in life might be confounded by current health and lifestyle. For example, obesity and smoking are known to adversely affect semen parameters, health, and life expectancy (20, 21). Medical conditions could also have an impact on semen quality; infertile men more often report symptoms of poor health, as measured by the Charlson comorbidity index (22). In addition, men with poor semen quality more often have hypertension and cardiovascular disease (15, 22, 23).

Based on the findings of previous studies, semen quality may represent not only a fertility marker (23, 24) but also a universal biomarker of later health and survival (13, 15). A long period of

follow-up is required to study such associations. Therefore, to address the possible long-term effects of low semen quality on morbidity, we studied the association between semen characteristics and subsequent hospitalizations in a large cohort of Danish men who attended a clinic for infertility assessment.

METHODS

Study population

The semen quality of 5,370 men was examined at the fertility clinic at Frederiksberg Hospital after referral for couple infertility by a general practitioner, urologist, or gynecologist from the Frederiksberg municipality in Copenhagen. Men who had semen analysis performed before 1977 or who were hospitalized before semen analysis ($n = 366$) were excluded. Men who died before the study ($n = 74$) and who emigrated ($n = 218$) were also excluded. We included 4,712 men with a first semen sample analyzed at Frederiksberg Hospital during 1977–2010. The data from these men were linked to records held in the Danish National Patient Registry by use of the personal identification number first given to all Danish citizens in 1968 and to all newborns and immigrants thereafter (25). The National Patient Registry was established in 1977 and holds information on all hospitalizations in Denmark (26). We recorded all first-time hospitalizations and used the *International Classification of Diseases* (ICD), eighth and tenth revisions, as the main diagnostic tools. Patient admissions were recorded until August 2015 or until death. A total of 181 men had no hospitalization recorded after the first semen sample, and they were followed until end of study, and 30 who died were followed until death, leaving 4,501 men who were hospitalized (Web Figure 1, available at https://academic.oup.com/aje). All incident diagnoses, along with their respective diagnosis codes, were allocated to 5 groups: all-cause diseases, cardiovascular diseases (ICD-8: 40199, 41009, 41099, 412009, 41299, 41309, 41399, 43091–43690, 45099–45503; ICD-10: DI009, DI019, DI050, DI10, DI109, DI15, DI110, DI119, DI119A, DI120, DI129, DI150–DI159, DI20, DI200, DI200A, DI200B, DI200C, DI208–DI209, DI21, DI120, DI210B, DI211, DI211A, DI211B, DI213–DI259, DI26, DI50, DI500–DI519, DI600–DI694, DI700-DI749), type 1 and 2 diabetes mellitus (ICD-8: 24900–24909, 25000–25101; ICD-10: DE10, DE100–DE109A, DE11, DE112–DE119A, DE133, DE139, DE140–DE149, DH360–DH360H), testicular cancer (ICD-8: 18699; ICD-10: DC620, DC621, DC629, DD401), and prostate cancer (ICD-8: 18599; ICD-10: DC619). The Ethical Committee for the Capital Region of Denmark approved the study in June 2011.

Analysis of semen samples

We used the results of the first semen sample the men had delivered for analysis due to couple infertility. Prior to delivery of these samples, the men were advised to keep an ejaculation abstinence period of 3–4 days. The actual abstinence period was 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 1 hour after ejaculation. The samples were kept at room temperature in the laboratory during the analysis. Semen volume was assessed by aspiration. For sperm motility assessment, a 10-μL drop of semen was placed on a glass slide, covered with a coverslip, and examined at 100× magnification. Spermatozoa were classified as motile or immotile. The sperm concentration was subsequently assessed using improved Neubauer hemocytometers. The laboratory at the fertility clinic Frederiksberg Hospital worked in close collaboration with the Copenhagen General Practitioners' Laboratory (KPLL) and followed the guidelines from quality control groups of the Nordic Association for Andrology (NAFA). Cutoff points and definitions of semen characteristics were done according to World Health Organization guidelines (27).

Statistical analysis

Semen volume, sperm concentration, and motility were outcome variables. Cutoff values for semen analysis followed the World Health Organization's most recently recommended lower reference values (28): sperm concentration <15 million/mL, semen volume <1.5 mL, total sperm count <40 million, and total motility ≤40%. Sperm concentration was categorized as 0, 1–15 million/mL, 16–40 million/mL, or >40 million/mL, and total sperm count as 0, 1–40 million, 41–120 million, or >120 million. We decided to use cutoff values rather than continuous modeling. In the initial descriptive analysis, we calculated the mean, standard deviation, median, and range for all semen characteristics. We calculated time from first semen analysis to first hospitalization or death and plotted the data using Kaplan-Meier survival estimation and diagrams. Restricted mean survival time (29) was estimated as a function of sperm concentration. This measure corresponds to the area under each separate survival curve for each sperm concentration and corresponds to expected hospitalization-free survival. Restricted mean survival time is unbiased up to a right censoring level of 30% (30). In the present study, the right censoring level was 4.4% in the analysis of all hospitalizations (i.e., 4.4% did not have a hospitalization by the end of the follow-up). Cox regression was used to analyze the associations of hospitalizations by diagnosis group and by semen characteristics. The analysis was adjusted for age and year of birth. In all analyses, the Cox proportional hazards assumption of proportional hazards was fulfilled. We used a score process test to test the assumption (31). Analyses were performed using the ASSESS statement in PROC PHREG (SAS, version 9.4; SAS Institute, Inc., Cary, North Carolina).

RESULTS

We studied 4,712 men with a maximal follow-up time of 38 years and a mean follow-up time of 6 years until first hospitalization. The average age at semen analysis was 34 years and the mean sperm concentration was 70 million/mL, with a median of 51 million/mL (range, 0–1,244 million/mL). The mean of total sperm count was 219 million, with a median of 157 million (range, 0–7,752 million), as shown in Table 1.

We found a significant dose-response association between poorer semen quality and all-cause hospitalizations, reflected in the Kaplan-Meier plot according to sperm concentration

Table 1. Characteristics of 4,712 Men Seen for Infertility, Frederiksberg Hospital, Denmark, 1977–2010

Characteristic	Sample Size	Mean (SD)	Median (Range)
Age at semen analysis, years	4,712	34 (7)	33 (16–85)
Year of semen analysis	4,712	1997 (9)	1998 (1977–2010)
Year of birth	4,712	1963 (10)	1963 (1918–1988)
Follow-up period, years	4,712	6 (7)	4 (0–38)
Semen volume, mL	4,481	3 (2)	3 (0–36)
Sperm concentration, million/mL	4,629	70 (73)	51 (0–1,244)
Total sperm count, millions	4,615	219 (262)	157 (0–7,752)
Motile sperm cells, %	4,089	41 (18)	38 (0–96)
Ejaculation abstinence, days	4,580	4 (3)	4(0–90)

Abbreviations: SD, standard deviation.

(Figure 1A). Similarly, we saw a significant association between sperm concentration above 40 million/mL and a low probability of hospitalizations for diabetes and cardiovascular disease (Figures 1B and 1C). Again, we saw the lowest probability for diabetes and cardiovascular disease for high levels of both total sperm count and motility but not for semen volume (Web Figure 2). The same pattern was found after adjustment for year of birth and age (Table 2). A 50% (95% confidence interval (CI): 1.4, 1.6) higher risk of hospitalization for all-cause disease and a 40% (95% CI: 1.2, 1.6) higher risk of cardiovascular disease were found for men with sperm concentration below 15 million/mL compared with men with a concentration above 40 million/mL. Men with azoospermia had a 40% higher risk of overall hospitalization (95% CI: 1.2, 1.5) and a 10% higher risk of hospitalization for cardiovascular disease (95% CI: 0.9, 1.4) compared with men with a sperm concentration above 40 million/mL. A higher risk of hospitalization was also found for the other semen characteristics, including motility and total sperm count. For semen volume and period of abstinence (results not shown), no association was found with hospitalization (Web Figure 2). Because of the low number of hospitalizations for diabetes ($n = 168$), testicular cancer ($n = 67$), and prostate cancer ($n = 63$), statistical modeling was not feasible for these outcomes (i.e., there were fewer than 10 observations in each category to be modeled).

Allocating the patients into smaller groups based on sperm concentration confirmed a clear dose-response association, such that higher levels of sperm concentration were related to decreased risk of first-time hospitalization with a significant 2-sided P for trend of <0.001 (Web Table 1). The relative risk for first-time hospitalization decreased by 0.029 (95% CI: 0.019, 0.040) for each 10 million/mL increase in sperm concentration when compared with the referent of a 30-year-old man born in 1955 who had a sperm concentration of 200 million/mL.

The mean time free of hospitalization showed a nearly linear increase with increasing sperm concentration up to 200 million/mL, after which it plateaued (Figure 2). When linear regression was applied for the interval of 0–200 million/mL, the remaining mean time free of hospitalization increased by 10 (standard error, 0.75) days for every 1 million/mL increase in sperm concentration, up to 200 million/mL. Men with a sperm concentration of 195–200 million/mL were, on average, hospitalized for the first time 7 years later than men with a sperm concentration of 0–5 million/mL (Figure 2). Mortality was higher for men with a sperm concentration below 15 million/mL when compared with men with higher sperm concentrations (Web Figure 3).

DISCUSSION

In this cohort study of 4,712 men, followed for up to 38 years, semen quality was associated with subsequent increased risk of hospitalization and increased mortality. A clear dose-response relationship between decreasing semen quality and increased risk for hospitalization was present, and the increased risk was found for a wide range of diseases, including cardiovascular disease, diabetes, testicular cancer, and prostate cancer. Men with the lowest semen quality had their first hospitalization on average 7 years earlier than men with the highest semen quality. This finding illustrates the public health impact of our findings and supports previous studies suggesting that semen quality is a strong biomarker for later health, morbidity, and mortality (13, 15).

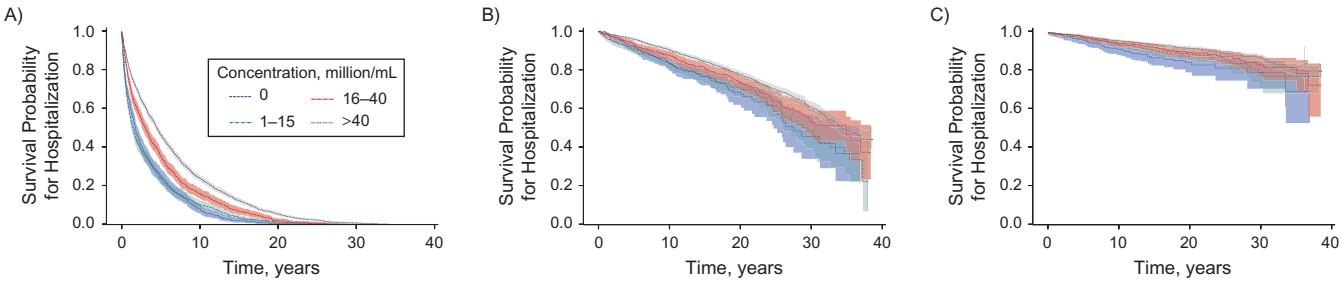

Figure 1. Annual survival probabilities among 4,712 men seen for infertility, Frederiksberg Hospital, Denmark, 1977–2010. Annual survival probabilities for first hospitalization following first semen analysis for all causes (A), cardiovascular diseases (B), and diabetes (C), according to sperm concentration. Shaded areas are 95% confidence intervals.

Table 2. Hazard Ratios for Hospitalization According to Sperm Concentration and Total Sperm Count and Type of Disease Among Men Seen for Infertility, Frederiksberg Hospital, Denmark, 1977–2010

Sperm Count and Concentration	No. of Participants	Unadjusted HR	95% CI	Adjusted HR	95% CI
All Causes (n = 4,501)[a]					
Sperm concentration, million/mL					
0	466	1.8	1.6, 2.0	1.4	1.2, 1.5
1–15	691	1.8	1.6, 1.9	1.5	1.4, 1.6
16–40	751	1.3	1.2, 1.4	1.1	1.0, 1.2
>40	2,511	1	Referent	1	Referent
Not available	82				
Total sperm count, millions					
0	454	1.9	1.7, 2.1	1.4	1.3, 1.6
1–40	615	1.8	1.6, 1.9	1.6	1.4, 1.7
41–120	829	1.3	1.2, 1.5	1.2	1.1, 1.3
>120	2,507	1	Referent	1	Referent
Not available	96				
Cardiovascular Diseases (n = 961)					
Sperm concentration, million/mL					
0	91	1.6	1.3, 1.9	1.1	0.9, 1.4
1–15	146	1.5	1.3, 1.8	1.4	1.2, 1.6
16–40	154	1.2	1.0, 1.4	1.1	1.0, 1.3
>40	549	1	Referent	1	Referent
Not available	21				
Total sperm count, millions					
0	86	1.6	1.3, 2.0	1.2	0.9, 1.4
1–40	133	1.6	1.4, 1.9	1.4	1.2, 1.7
41–120	190	1.4	1.2, 1.6	1.3	1.1, 1.5
>120	525	1	Referent	1	Referent
Not available	27				
Diabetes (n = 168)[b]					
Sperm concentration, million/mL					
0	21	1.9	1.4, 2.5		
1–15	16	1.4	1.1, 1.8		
16–40	28	1.2	0.9, 1.6		
>40	99	1	Referent		
Not available	4				
Total sperm count, millions					
0	21	2.2	1.6, 2.9		
1–40	21	1.8	1.4, 2.3		
41–120	38	1.4	1.1, 1.7		
>120	84	1	Referent		
Not available	4				
Testicular Cancer (n = 67)[b]					
Sperm concentration, million/mL					
0	9	2.0	1.4, 2.7		
1–15	9	1.6	1.2, 2.2		
16–40	10	1.2	0.9, 1.6		
>40	35	1	Referent		
Not available	4				

Table continues

Table 2. Continued

Sperm Count and Concentration	No. of Participants	Unadjusted HR	95% CI	Adjusted HR	95% CI
Total sperm count, millions					
0	9	2.1	1.6, 3.0		
1–40	8	1.9	1.4, 2.5		
41–120	14	1.3	1.0, 1.7		
>120	32	1	Referent		
Not available	4				
	Prostate Cancer (n = 63)[b]				
Sperm concentration, million/mL					
0	9	2.1	1.5, 2.9		
1–15	2	1.5	1.1, 2.1		
16–40	11	1.3	1.0, 1.7		
>40	39	1	Referent		
Not available	2				
Total sperm count, millions					
0	9	2.2	1.6, 3.1		
1–40	4	1.8	1.3, 2.4		
41–120	12	1.3	1.0, 1.7		
>120	36	1	Referent		
Not available	2				

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Of the initial sample of 4,712 men, 30 men died and 181 men had no hospitalization data available after their first semen sample.

[b] Because of low numbers of hospitalizations, adjustment was statistically not feasible for diabetes, prostate cancer, and testicular cancer.

Our study supports previous findings in studies that examined the relationship between semen quality and subsequent morbidity and mortality. A Danish cohort study of 43,277 men, with a follow-up time of up to 40 years, found a dose-response relationship between increasing sperm concentration and decreasing mortality, with approximately 45% lower mortality for men with a sperm concentration above 40 million/mL (13). In the same study, the analysis was stratified by fertility status, and men with children, before or after semen analysis, had lower mortality than did childless men (standard mortality ratio = 1.89, 95% CI: 1.67, 2.14) (13). In a cohort study of 11,935 infertile men, of whom 69 died (0.58%), the group with a sperm concentration below 15 million/mL had a 2.2 times higher mortality rate, compared with the group with sperm concentration above 15 million/mL (14). A newly published US study found higher morbidity among 13,027 men with male factor infertility than among 23,860 who were only tested for infertility (15); however, no semen quality data were included. In the latter study, the follow-up time was 9 years, and investigators found that having an infertility diagnosis increased the risk of developing diabetes or ischemic heart disease by 30% and 48%, respectively (15). Our findings are in accordance with these results and support the suggestion of higher hospitalization risk for subfertile men.

If semen quality is a universal biomarker for overall health, it could be mediated through the male genome—approximately 15% of the genome is involved in reproduction (16). In addition, low semen quality could be caused by lifestyle and health factors, such as current health, smoking status, and body mass index, which are known to affect semen quality as well as morbidity and mortality (20, 21, 32–34). Semen quality is also associated with circulating levels of testosterone, which predict future morbidity and mortality as found in infertile men, suggesting impaired Leydig cell function (34–36). An association between low testosterone levels and risk of cardiovascular disease has also been found in several studies (37–39). A major review by Araujo et al. (17) confirmed that low endogenous levels of testosterone were associated with increased risk of mortality and cardiovascular-related mortality. Meeker et al. (40) found a positive association between sperm concentration and testosterone levels. In addition, infertile men are more likely to develop cancers, especially testicular cancer. In a Danish cohort study of 32,442 infertile men, a 2- to 3-fold increased risk of testicular cancer risk was found among men with a sperm concentration below 20 million/mL, suggesting common risk factors for impaired spermatogenesis and testicular cancer (7). Our study supports this association, with a finding of a hazard ratio for testicular cancer of 1.6 among participants with a sperm concentration of 1–15 million/mL.

In addition, studies suggest that in utero exposure to lifestyle factors or chemicals with endocrine-disrupting abilities could explain the link between men's reproductive and somatic health (40–42). Maldescent of testis, hypospadias, and testicular cancer

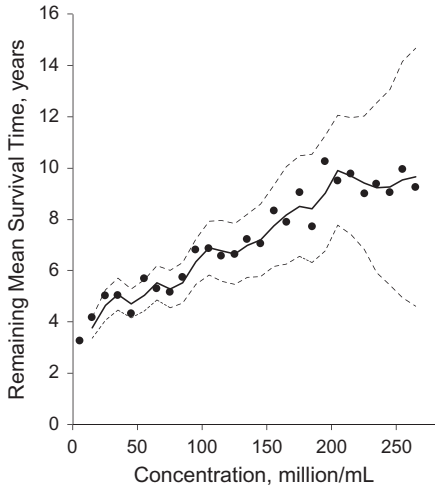

Figure 2. Remaining mean survival time among 4,712 men seen for infertility, Frederiksberg Hospital, Denmark, 1977–2010. Remaining mean survival time and 95% confidence intervals for first-time hospitalizations according to sperm concentrations. For concentrations from 0–200 million/mL, there seems to be a linear association.

have been found to be increased with decrease in semen quality (5, 7, 43–46). Coherency has been found between the above-mentioned conditions and diseases, because they are reciprocal risk factors for each other, are developed in the embryonic stage, and are different manifestations of an underlying testicular dysgenesis syndrome (43, 47). If these in utero factors may result in diseases, such as cardiovascular disease in later life, and at the same time are related to semen quality, one could expect to find a link between semen quality and disease occurrence in men. In our study, such an association was observed between low sperm quality and higher risk of hospitalization in a dose-response manner, not only for cardiovascular disease and diabetes, but also for all-cause hospitalizations.

Limitations and strengths

A major strength of the present study is the large study population (n = 4,712), the comprehensive follow-up (because individuals could be tracked in the high-quality Danish registry), and the long-term follow-up. Also, overall mortality was less than 1%, which meant that the influence of competing risk was low. Additionally, the same technician analyzed the semen samples through the entire study period, and the same methods were used for all semen samples. The cohort study approach used and the adjustment for age and cohort further support the hypothesis of causality between low semen quality and poor health.

The men included in the present study had been referred for infertility treatment and might not represent the general population. Couples seeking fertility treatment in the 1970s and 1980s had a relatively higher socioeconomic status, and usually the men who seek infertility treatment are married, are older, and have a higher educational level than the rest of the population (8, 48). Higher socioeconomic status would be expected to confer lower morbidity (13, 49), and we would thus expect lower levels of morbidity and mortality in the men included in our study, which could introduce a selection bias. Although our sample was probably not representative of the general population, we found a strong association between semen quality and later risk of hospitalization, making it therefore less important whether the men represent the general population. Because the study was register-based, we were not able to obtain information about social status, fertility, and lifestyle, including smoking and dietary habits, all of which could be possible confounders for the observed associations. In addition, a larger study cohort would have provided the opportunity to investigate hospitalization for specific diseases in more detail.

Conclusion

We found that semen quality is a strong universal health biomarker. We were able to show that men with low semen quality more often required hospitalization, in particular for diabetes mellitus and cardiovascular disease. To our knowledge, this study is the first to correlate semen quality and subsequent risk of hospitalization on a long timescale. However, our findings should be further investigated and confirmed by other studies.

ACKNOWLEDGMENTS

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Conflict of interest: none declared.

REFERENCES

1. Louis JF, Thoma ME, Sørensen DN, et al. The prevalence of couple infertility in the United States from a male perspective: evidence from a nationally representative sample. *Andrology*. 2013;1(5):741–748.

2. Bonde JP, Ernst E, Jensen TK, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet*. 1998;352(9135):1172–1177.
3. Jensen TK, Carlsen E, Jørgensen N, et al. Poor semen quality may contribute to recent decline in fertility rates. *Hum Reprod*. 2002;17(6):1437–1440.
4. Jørgensen N, Asklund C, Carlsen E, et al. Coordinated European investigations of semen quality: results from studies of Scandinavian young men is a matter of concern. *Int J Androl*. 2006;29(1):54–61.
5. Auger J, Kunstmann JM, Czyglik F, et al. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med*. 1995;332(5):281–285.
6. Walsh TJ, Croughan MS, Schembri M, et al. Increased risk of testicular germ cell cancer among infertile men. *Arch Intern Med*. 2009;169(4):351–356.
7. Jacobsen R, Bostofte E, Engholm G, et al. Risk of testicular cancer in men with abnormal semen characteristics: cohort study. *BMJ*. 2000;321(7264):789–792.
8. Eisenberg ML, Betts P, Herder D, et al. Increased risk of cancer among azoospermic men. *Fertil Steril*. 2013;100(3):681–685.
9. Eisenberg ML, Li S, Brooks JD, et al. Increased risk of cancer in infertile men: analysis of US claims data. *J Urol*. 2015; 193(5):1596–1601.
10. Peng X, Zeng X, Peng S, et al. The association risk of male subfertility and testicular cancer: a systematic review. *PLoS One*. 2009;4(5):e5591.
11. Giwercman A, Richiardi L, Kaijser M, et al. Reduced risk of prostate cancer in men who are childless as compared to those who have fathered a child: a population based case-control study. *Int J Cancer*. 2005;115(6):994–997.
12. Walsh TJ, Schembri M, Turek PJ, et al. Increased risk of high-grade prostate cancer among infertile men. *Cancer*. 2010; 116(9):2140–2147.
13. Jensen TK, Jacobsen R, Christensen K, et al. Good semen quality and life expectancy: a cohort study of 43,277 men. *Am J Epidemiol*. 2009;170(5):559–565.
14. Eisenberg ML, Li S, Behr B, et al. Semen quality, infertility and mortality in the USA. *Hum Reprod*. 2014;29(7): 1567–1574.
15. Eisenberg ML, Li S, Cullen MR, et al. Increased risk of incident chronic medical conditions in infertile men: analysis of United States claims data. *Fertil Steril*. 2016;105(3): 629–636.
16. Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. *Nat Med*. 2008;14(11): 1197–1213.
17. Araujo AB, Dixon JM, Suarez EA, et al. Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2011; 96(10):3007–3019.
18. Haring R, Volzke H, Steveling A, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79. *Eur Heart J*. 2010;31(12):1494–1501.
19. Chock B, Lin TC, Li CS, et al. Plasma testosterone is associated with Framingham risk score. *Aging Male*. 2012; 15(3):134–139.
20. Jensen TK, Andersson AM, Jørgensen N, et al. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril*. 2004;82(4):863–870.
21. Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, et al. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod*. 2007;22(1):188–196.
22. Eisenberg ML, Li S, Behr B, et al. Relationship between semen production and medical comorbidity. *Fertil Steril*. 2015; 103(1):66–71.
23. Salonia A, Matloob R, Gallina A, et al. Are infertile men less healthy than fertile men? Results of a prospective case-control survey. *Eur Urol*. 2009;56(6):1025–1031.
24. Ventimiglia E, Capogrosso P, Boeri L, et al. Infertility as a proxy of general male health: results of a cross-sectional survey. *Fertil Steril*. 2015;104(1):48–55.
25. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol*. 2014;29(8):541–549.
26. Schmidt M, Schmidt SA, Sandegaard JL, et al. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol*. 2015;7:449–490.
27. Department of Reproductive Health and Research, *WHO Laboratory Manual for the Examination and Processing of Human Semen.* 5th ed. Geneva, Switzerland: World Health Organization; 2010, 223–224.
28. Cooper TG, Noonan E, von ES, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update*. 2010;16(3):231–245.
29. Royston P, Parmar MK. Restricted mean survival time: an alternative to the hazard ratio for the design and analysis of randomized trials with a time-to-event outcome. *BMC Med Res Methodol*. 2013;13:152.
30. Zhong M, Hess KR. *Mean Survival Time from Right Censored Data*. Berkeley, CA: The Berkeley Electronic Press; 2009. (Report No. 66).
31. Lin DY, Wei LJ, Ying Z. Checking the Cox model with cumulative sums of martingale-based residuals. *Biometrika*. 1993;80(3):557–572.
32. Jensen TK, Jørgensen N, Asklund C, et al. Self-rated health and semen quality among 3,457 young Danish men. *Fertil Steril*. 2007;88(5):1366–1373.
33. Hammoud AO, Wilde N, Gibson M, et al. Male obesity and alteration in sperm parameters. *Fertil Steril*. 2008;90(6): 2222–2225.
34. Aggerholm AS, Thulstrup AM, Toft G, et al. Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertil Steril*. 2008;90(3):619–626.
35. Andersson AM, Jørgensen N, Frydelund-Larsen L, et al. Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. *J Clin Endocrinol Metab*. 2004;89(7):3161–3167.
36. Kilcoyne KR, Smith LB, Atanassova N, et al. Fetal programming of adult Leydig cell function by androgenic effects on stem/progenitor cells. *Proc Natl Acad Sci USA*. 2014;111(18):E1924–E1932.
37. Laaksonen DE, Niskanen L, Punnonen K, et al. Testosterone and sex hormone–binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care*. 2004;27(5):1036–1041.
38. Oskui PM, French WJ, Herring MJ, et al. Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *J Am Heart Assoc*. 2013;2(6):e000272.
39. Stellato RK, Feldman HA, Hamdy O, et al. Testosterone, sex hormone–binding globulin, and the development of type 2 diabetes in middle-aged men: prospective results from the Massachusetts male aging study. *Diabetes Care*. 2000;23(4): 490–494.
40. Meeker JD, Godfrey-Bailey L, Hauser R. Relationships between serum hormone levels and semen quality among men from an infertility clinic. *J Androl*. 2007;28(3):397–406.

41. Phillips KP, Tanphaichitr N. Human exposure to endocrine disrupters and semen quality. *J Toxicol Environ Health B Crit Rev*. 2008;11(3-4):188–220.
42. Phillips KP, Foster WG. Endocrine toxicants with emphasis on human health risks. *J Toxicol Environ Health B Crit Rev*. 2008; 11(3-4):149–151.
43. Virtanen HE, Rajpert-De Meyts E, Main KM, et al. Testicular dysgenesis syndrome and the development and occurrence of male reproductive disorders. *Toxicol Appl Pharmacol*. 2005; 207(2 suppl):501–505.
44. Carlsen E, Giwercman A, Keiding N, et al. Evidence for decreasing quality of semen during past 50 years. *BMJ*. 1992; 305(6854):609–6013.
45. Toppari J, Kaleva M, Virtanen HE. Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. *Hum Reprod Update*. 2001; 7(3):282–286.
46. Møller H, Skakkebaek NE. Risk of testicular cancer in subfertile men: case-control study. *BMJ*. 1999;318(7183): 559–562.
47. Skakkebaek NE. Testicular dysgenesis syndrome: new epidemiological evidence. *Int J Androl*. 2004;27(4):189–191.
48. Hotaling JM, Davenport MT, Eisenberg ML, et al. Men who seek infertility care may not represent the general US population: data from the National Survey of Family Growth. *Urology*. 2012;79(1):123–127.
49. Lantz PM, House JS, Lepkowski JM, et al. Socioeconomic factors, health behaviors, and mortality: results from a nationally representative prospective study of US adults. *JAMA*. 1998;279(21):1703–1708.