Human Reproduction, Vol.34, No.11, pp. 2266–2273, 2019
Advance Access Publication on November 4, 2019 doi:10.1093/humrep/dez189

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Male factor infertility and risk of death: a nationwide record-linkage study

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Submitted on May 13, 2019; resubmitted on August 5, 2019; editorial decision on August 8, 2019

STUDY QUESTION: What is the risk of death among men with oligospermia, unspecified male factor and azoospermia in the years following fertility treatment?

SUMMARY ANSWER: No significantly elevated risk was observed among men with oligospermia and unspecified male factor, while an increased risk was found among men with azoospermia.

WHAT IS KNOWN ALREADY: Previous studies have shown associations between male factor infertility and risk of death, but these studies have relied on internal reference groups and the risk of death according to type of male infertility is not well characterized.

STUDY DESIGN, SIZE, DURATION: In this prospective record-linkage cohort study, we identified men who had undergone medically assisted reproduction (MAR) between 1994 and 2015. Data was linked to the Danish causes of death register and sociodemographic registers through personal identification numbers assigned to all Danish citizens at birth.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Men that had undergone MAR in Denmark (MAR Cohort; $n = 64\,563$) were identified from the Danish IVF register, which includes data on whether infertility was due to male factor. For each man in the MAR cohort, five age-matched men who became fathers without fertility treatment were selected from the general population (non-MAR fathers; $n = 322\,108$). Men that could not adequately be tracked in the Danish CPR register ($n = 1259$) and those that were censored prior to study entry ($n = 993$) were excluded, leaving a final population of 384 419 men. Risk of death was calculated by Cox regression analysis with age as an underlying timeline and adjustments for educational attainment, civil status and year of study entry. The risk of death was compared among men with and without male factor infertility identified from the IVF register (internal comparisons) as well as to the non-MAR fathers (external comparison).

MAIN RESULTS AND THE ROLE OF CHANCE: The risk of death between the MAR cohort (all men, regardless of infertility) and the non-MAR fathers was comparable [hazard ratio (HR), 1.07; 95% CI, 0.98–1.15]. When the MAR cohort was limited to infertile men, these men were at increased risk of death [HR, 1.27; 95% CI, 1.12–1.44]. However, when stratified by type of male factor infertility, men with azoospermia had the highest risk of death, which persisted when in both the internal [HR, 2.30; 95% CI, 1.54–3.41] and external comparison [HR, 3.32; 95% CI, 2.02–5.40]. No significantly elevated risk of death was observed among men with oligospermia [HR, 1.14; 95% CI, 0.87–1.50] and unspecified male factor [HR, 1.10; 95% CI, 0.75–1.61] compared with the non-MAR fathers. The same trends were observed for the internal comparison.

LIMITATIONS, REASONS FOR CAUTION: Duration of the follow-up was limited and there is limited generalizability to infertile men who do not seek fertility treatment.

WIDER IMPLICATIONS OF THE FINDINGS: Using national health registers, we found an increased risk of death among azoospermic men while no increased risk was found among men with other types of infertility. For the azoospermic men, further insight into causal pathways is needed to identify options for monitoring and prevention.

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STUDY FUNDING/COMPETING INTEREST(S): This study is part of the ReproUnion collaborative study, co-financed by the European Union, Interreg V ÖKS. C.G.'s research stay at Stanford was funded by grants from the University of Copenhagen, Kong Christian den Tiendes Fond, Torben og Alice Frimodt Fond and Julie Von Müllen Fond. M.E. is an advisor for Sandstone and Dadi. All other authors declare no conflict of interests.

TRIAL REGISTRATION NUMBER: Not relevant.

Key words: male factor infertility / mortality / azoospermia / oligospermia / register-based record linkage study / medically assisted reproduction / epidemiology / infertility

Introduction

In the developed countries, infertility is among the most prevalent chronic disease in the age group 25–44 years affecting up to 15% of couples trying to become parents (Nielsen *et al.*, 2016). In fact, nearly 10% of all Danish children were conceived after fertility treatment in 2018 (Danish Fertility Society, 2018), which is higher than any other European country. Although increasing maternal age inevitably plays a role, male factor infertility, defined as poor semen quality, serves as a sole or contributing factor in up to half of cases. As modern fertility treatment focuses on obtaining gametes for assisted reproduction, a thorough evaluation of the man is sometimes a missed opportunity. However, data has shown that men with impaired fertility may face additional health problems in the years following fertility treatment. In recent years, prospective studies from Europe and the USA have shown that infertile men have higher risks of cardiovascular disease (Eisenberg *et al.*, 2016), certain cancers (Eisenberg *et al.*, 2013), autoimmune diseases (Brubaker *et al.*, 2018, Glazer *et al.*, 2017b), diabetes (Eisenberg *et al.*, 2016, Glazer *et al.*, 2017a) and overall hospitalizations (Latif *et al.*, 2017). In addition, cross-sectional studies have shown that infertile men present with more comorbidities than their fertile peers (Salonia *et al.*, 2009) including higher rates of hypogonadism, metabolic abnormalities and poor bone mineral density (Bobjer *et al.*, 2016). The underlying links remain unknown, but is has been hypothesized that common etiologies, such as hypogonadism or genetics, might explain the association.

However, data on death rates among infertile men are few and lack nuances pertaining to the risk related to specific types of infertility, including oligospermia (reduced semen quality) and azoospermia (complete lack of sperm cells in the ejaculate). One US study based on semen samples from 11 935 men found higher death rates among men with impaired semen parameters when compared with men with normal parameters (Eisenberg *et al.*, 2014). However, as this study compared men with semen concentrations above and below 15 mill/ml it is uncertain to what degree the azoospermic men may have influenced the risk estimates. Another study based on semen samples from 43 277 Danish men similarly linked impaired semen parameters to higher death rates (Jensen *et al.*, 2009). However, this study excluded azoospermic men from the final analysis.

Furthermore, both studies mentioned above relied on internal reference groups to determine the risk of death. Such results should be verified by inclusion of a population-based comparison group as reports on the female side have shown that women in fertility treatment generally have a lower risk of death (Vassard *et al.*, 2018). However, whether this also applies to the male population is uncertain.

The aim of this study was to determine whether men, with different types of male factor infertility, were at increased risk of death in the years following fertility treatment. Our nationwide study included men identified from all fertility clinics in Denmark and a population-based comparison group of age-matched men who became fathers without fertility treatment.

Materials and Methods

Setting

In Denmark, fertility treatment is public and tax-financed among childless women/couples if the woman is under 41 years old, which means that fertility treatment is free of charge for up to three fresh IVF cycles and an unlimited number of IUI cycles (in practice three to six cycles). Further, fertility treatment is offered in the private health care sector for women under 46 years old. Overall, 50% of all medically assisted reproduction (MAR) treatments are provided in the public health care system. All fertility clinics in Denmark adhere to national guidelines provided by the Danish Fertility Society to ensure compatibility between the clinics (Danish Fertility Society, 2019). As part of the male evaluation, a semen sample is provided by masturbation in sterile containers and the men are carefully instructed regarding abstinence time. If the first sample is abnormal, the men must provide a second sample. All samples were analyzed according to the World Health Organization (WHO) guidelines with the following normative reference values: before 2010 sperm concentration 20×10^6/ml, semen volume 2.0 ml, motile sperm 50%, morphology 15% and total sperm count 40×10^6, and from 2010 sperm concentration 15×10^6/ml, semen volume 1.5 ml, motile sperm 40%, morphology 4% and total sperm count 39×10^6 (Cooper *et al.*, 2010, WHO, 1999). The absence of spermatozoa in two consecutive samples indicated azoospermia (WHO, 2010).

Study population

MAR cohort

We identified a cohort of men who had undergone any type of MAR treatment from all public or private fertility clinics in Denmark during 1994–2015. This information is available from the national Danish IVF Register, which was established in 1994 and includes information on whether infertility was due to male factor (Andersen *et al.*, 1999). The register was updated in 2006 with a more detailed documentation regarding male factor infertility, which we previously have described (Glazer *et al.*, 2017a). In brief, the male factor variable was initially recorded as 'yes' or 'no', and from 2006 onwards as International Classification of Disease (ICD) 10 diagnosis codes (aspermia N469A, azoospermia N469B, oligospermia N469C, oligo-teratozoospermia N469D, other reasons for male infertility N469W, male infertility

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Table I Baseline characteristics of the external group and the MAR cohort.

	Non-MAR fathers ($n = 320\,042$)	MAR Cohort ($n = 64\,377$)	Male factor ($n = 24\,062$)	Azoospermic[a] ($n = 1906$)
Median age at baseline (years)	33.3	33.8	33.9	34.4
Median follow-up time (years)	9.7	9.2	9.7	8.4
Median age at death (years)	47.0	47.7	47.5	45.1
Educational level, *n*(%)				
Less than high school	55 360 (17.7)	8827 (14.3)	3424 (14.8)	339 (18.4)
High school or equivalent	23 233 (7.4)	4645 (7.5)	1762 (7.6)	116 (6.3)
Skilled workers	143 564 (46.0)	29 485 (47.7)	10 925 (47.1)	967 (52.5)
Bachelor or equivalent	45 505 (14.6)	9553 (15.4)	3563 (15.4)	209 (11.3)
Higher University	44 150 (14.2)	9358 (15.1)	3519 (15.2)	211 (11.4)
Missing	10 739			
Civil Status, *n* (%)				
Married/legal partnership	170 776 (53.5)	33 466 (52.0)	12 513 (52.1)	971 (51.0)
Divorced	16 177 (5.1)	3752 (5.8)	1594 (6.6)	216 (11.3)
Unmarried	132 516 (41.5)	27 100 (42.1)	9924 (41.3)	717 (37.7)
Missing	632			

[a]Data on azoospermic men were available for the IVF register period covering 2006–2015.
MAR: medically assisted reproduction

Table II Distribution of death causes in the MAR cohort and the non-MAR fathers ($n = 3604$ men).

	Non-MAR fathers *n* (%)	MAR cohort *n* (%)	Male factor *n* (%)	Azoospermic *n* (%)
Cancer				
	952 (31.7)	201 (33.2)	97 (37.0)	11 (37.9)
Circulatory diseases				
	536 (17.9)	124 (20.5)	47 (17.9)	5 (17.2)
Intentional self-harm				
	373 (12.4)	70 (11.6)	23 (8.8)	<5
External causes[a]				
	445 (14.8)	59 (9.7)	18 (6.9)	<5
Mental and behavioral disorders[b]				
	125 (4.2)	21 (3.5)	13 (5.0)	<5
Other diseases[c]				
	567 (18.9)	131 (21.6)	64 (24.4)	5 (17.2)
Total deaths	2998	606	262	29

Causes of deaths were available from 1994–2016 and based on International Classification of Diseases (ICD)-10 codes.
Table is not age-adjusted as the groups were age-matched.
[a]Accidents (V01-X59), assault (X85-Y09), and other external causes (Y00-Y98).
[b]Includes ICD-10 (F00–99). Mainly substance abuse.
[c]Includes all ICD-10 codes excluding cancers (C00–97), mental and behavioral disorders (F00–99), intentional self-harm (ICD-10:X60–84), and external causes.

unspecified N469X, female infertility due to male factor N974). Those without male factor infertility had a diagnosis code of normal semen quality (EZDH01) or were sterilized (Z302). If no diagnosis code was registered at the first visit, the code of their second visit was used.

We grouped men with a diagnosis code of infertility into three categories: azoospermia included men with aspermia and azoospermia, oligospermia included men with oligospermia and oligoteratozoospermia and unspecified male factor included men with unspecified male factor, male infertility due to 'other causes' and female infertility due to male factor, if the man was not sterilized.

Non-MAR fathers

For each man identified in the IVF register ($n = 64\,563$), five age-matched fathers were individually matched from the Danish medical

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birth register ($n = 322\,108$), which records all births in Denmark (Bliddal *et al.*, 2018). These men became fathers within +/−2.5 years from the date their matched MAR case entered fertility treatment without appearing in the IVF register (non-MAR fathers). This narrow inclusion time was chosen to ensure that all men were trying to conceive within the same time period.

Linkage to national registers

Both groups were linked to national health registers through unique identification numbers (civil registration numbers, CPR) assigned to all Danish citizens at birth. Information on death during follow-up was obtained through the Danish register of Causes of Death (Helweg-Larsen, 2011) (available until Dec 2016) and the Danish CPR register (available until May 2018) (Pedersen, 2011). Men that could not adequately be tracked in the Danish CPR register ($n = 1259$; e.g. men without a registered code) and those that were censored prior to study entry ($n = 993$; e.g. men that emigrated prior to study entry) were excluded, leaving a final population of 384 419 men. For the stratified analysis regarding type of male factor infertility, men with missing diagnosis codes of infertility ($n = 13\,228$) were excluded.

Covariates

Civil status (married/legal partnership, divorced or unmarried) and educational attainment (less than high school, high school, skilled workers, bachelor's degree or higher university degree) were extracted at year of study entry (MAR cohort = entry year of fertility treatment, non-MAR fathers = year of child's birthdate) and were available from the Danish CPR register and Statistics Denmark.

Statistical analysis

External comparison

First, we compared the risk of death between the MAR cohort (all men, regardless of male factor infertility) and the non-MAR fathers. Second, this analysis was repeated including only men with male factor infertility. Third, the risk of death according to type of male factor infertility in comparison to the non-MAR fathers was assessed. For all the above analyses, the MAR cohort was exclusively matched to their respective non-MAR fathers.

Internal comparison

Next, we compared the risk of death among men with male factor infertility to those without male factor infertility from the MAR cohort. The risk of death according to type of male factor infertility in comparison to men without male factor infertility was also conducted. Also, the internal comparisons were repeated for excluding men with a history of cancer ($n = 1318$), as cancer (or its treatment) may be the root cause of poor semen quality and represent the reason for premature death. Cancer diagnoses were not available for the non-MAR fathers and therefore were only assessed in the internal comparison.

Finally, we assessed the influence of competing risks of death from external causes (e.g. accidents, assault) as a sensitivity analyses for both the external and internal analysis. The hazard ratio (HR) of death was calculated using Cox proportional hazards models with age as the underlying time scale. The men were considered at risk from study entry and were followed until death, emigration, disappearance or end of follow-up on 22 May 2018, whichever came first. The crude analysis was age adjusted as it was inherent in the model. Further adjustments are presented in the tables. Kaplan–Meier plots allowed for proportional hazard assumption. The results are expressed as HRs with corresponding 95% CIs. Analyses and data management were conducted in SAS (version 9.4; SAS Institute, Cary, NC, USA).

Approval

The study was approved by Danish Data Protection Agency J. no.: BFH-2015-091. According to the Danish legislation, register-based studies do not require ethical approval as these studies do not involve direct contact with individuals.

Results

The baseline characteristics are presented in Table I. The causes of deaths were available among 3604 men (until December 2016) and are presented in Table II. Deaths from external causes were more prevalent among the non-MAR fathers than the MAR cohort (14.8% versus 9.7%). Cancer was the most prevalent cause of death (32%).

The comparisons of death rates between the MAR cohort and the non-MAR fathers are presented in Table III. The risk of death between the MAR cohort (all men, regardless of male factor infertility) and the non-MAR fathers was comparable [HR, 1.07; 95% CI, 0.98–1.15]. However, when we limited the MAR cohort to those with male factor infertility, these men were at an increased risk [HR, 1.27; 95% CI, 1.12–1.44]. When stratified by type of male factor infertility, the azoospermic men had the highest risk [HR, 3.32; 95% CI, 2.02–5.40] compared with the non-MAR fathers. Oligospermic men and those with unspecified male factor infertility had risk estimates of HR of 1.14 [95% CI, 0.87–1.50] and HR of 1.10 [95% CI, 0.75–1.61], respectively, when compared to non-MAR fathers.

The internal comparisons of death rates between men with and without male factor infertility are presented in Table IV. The increased risk of death among azoospermic men persisted [HR, 2.30; 95% CI, 1.54–3.41], although it was somewhat mitigated. However, the risk remained largely unchanged when the azoospermic men were compared with oligospermic men. Men with oligospermia and those with unspecified male factor did not appear to have an increased risk of death compared to men without male factor infertility. Excluding men with a history of cancer decreased the risk of death for azoospermic men to some degree [HR, 1.94; 95% CI, 1.23–3.05, not shown in tables]. Testing for competing risks from deaths of external causes increased risk estimates across almost all analyses (Tables III and IV; HR[b]).

Discussion

This nationwide cohort study of 384 419 men determined the risk of death among men that had undergone fertility treatment in Denmark. We found that men with azoospermia had an increased risk of death while no significantly increased risk was found among men with oligospermia and unspecified male factor. These findings were robust as they persisted in both the internal and external comparisons. Most men died of biological causes with cancer being the most prevalent cause of death. We hypothesize that the higher risk of death among

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Table III Comparison of death rates between the MAR cohort and the non-MAR fathers. HR: hazard ratio.

	n	Deaths	HR crude	HR[a]	HR[a,b]
MAR cohort versus non-MAR fathers					
Non-MAR fathers: All men	320 042	3798	ref [1.00]	ref [1.00]	ref [1.00]
MAR Cohort: All men	64 377	753	0.99 [0.92–1.07]	1.07 [0.98–1.15]	1.12 [1.03–1.21]
non-MAR fathers: Male factor infertility matches[c]	119 231	1384	ref [1.00]	ref [1.00]	ref [1.00]
MAR cohort: Male factor infertility[c,d]	24 062	327	1.18 [1.05–1.33]	1.27 [1.12–1.44]	1.35 [1.19–1.54]
Type of male factor infertility versus non-MAR fathers (available since 2006)					
Non-MAR fathers: Azoospermia matches[c]	9422	55	ref [1.00]	ref [1.00]	ref [1.00]
Azoospermia[e]	1906	34	3.05 [1.98–4.71]	3.32 [2.05–5.40]	3.66 [2.18–6.16]
Non-MAR fathers: Oligospermia matches[c]	63 363	335	ref [1.00]	ref [1.00]	ref [1.00]
Oligospermia[f]	12 813	72	1.08 [0.84–1.40]	1.14 [0.87–1.50]	1.26 [0.95–1.66]
Non-MAR fathers: Unspecified male factor matches[c]	17 876	180	ref [1.00]	ref [1.00]	ref [1.00]
Unspecified male factor[g]	3610	38	1.06 [0.75–1.51]	1.10 [0.75–1.61]	1.07 [0.71–1.61]

[a]Adjusted for educational attainment, civil status, and year of study entry.
[b]Competing risk analysis incorporating deaths from external causes as a competing risk.
[c]The term 'matches' refers to the matched men of Non-MAR fathers (reference group for each below analysis).
[d]Includes male factor = 'yes' from the first IVF register ($n = 5733$) and men with a diagnosis code of male factor infertility from the second register ($n = 18\,329$).
[e]Includes men with aspermia ($n = 165$).
[f]Includes men with oligo-teratozoospermia ($n = 5317$).
[g]Includes men with 'other reasons for infertility' ($n = 1170$) and men with unspecified male factor ($n = 2440$).

Table IV The internal comparisons of death rates between men with and without male factor infertility identified from the MAR cohort.

	n	Deaths	HR crude	HR[a]	HR[a,b]
Male factor versus no male factor					
No male factor infertility[c]	27 087	345	ref [1.00]	ref [1.00]	ref [1.00]
Male factor infertility	24 062	327	1.17 [1.00–1.36]	1.26 [1.07–1.48]	1.31 [1.11–1.52]
Type of male factor versus no male factor (available since 2006)					
Normal semen quality/sterilized	18 362	99	ref [1.00]	ref [1.00]	ref [1.00]
Azoospermia[d]	1906	34	2.28 [1.54–3.38]	2.30 [1.54–3.41]	2.40 [1.57–3.67]
Oligospermia[e]	12 813	72	0.96 [0.72–1.31]	0.97 [0.71–1.33]	1.08 [0.77–1.50]
Unspecified male factor[f]	3610	38	1.12 [0.77–1.64]	1.18 [0.78–1.78]	1.21 [0.77–1.89]

[a]Adjusted for educational attainment, civil status and year of study entry.
[b]Competing risk analysis incorporating deaths from external causes as competing risk.
[c]Includes men with male factor = 'no' from the first register ($n = 8725$) and men with without male factor infertility ($n = 18\,029$) or those sterilized in the second register ($n = 333$).
[d]Includes men with aspermia ($n = 165$).
[e]Includes men with oligo-teratozoospermia ($n = 5317$).
[f]Includes men with 'other reasons for infertility' ($n = 1170$) and men with unspecified male factor ($n = 2440$).

azoospermic men is due to common underlying etiologies, which might be associated with impaired fertility and risk of death.

The first study to compare the risk of death among infertile and fertile men was a German cohort of 601 men who provided a semen sample as part of an andrological evaluation (Groos *et al.*, 2006). This study found a possible association among oligospermic men, but only for a subset of men born between 1892 and 1931. However, as this study included men affected by World War II in Germany, extrapolation of such results is uncertain. Another Danish study of 43 277 men without azoospermia found higher death rates among men with lower semen quality suggesting that semen quality may serve as biomarker of health (Jensen *et al.*, 2009). The study was age- and period-adjusted but lacked adjustments for both civil status and educational attainment, which are both factors that may affect longevity (Johnson *et al.*, 2000, Montez *et al.*, 2012). Finally, one US study of 11 935 men evaluated for infertility found higher death rates among men with impaired semen parameters (for instance, sperm concentrations <15 mill/ml) (Eisenberg *et al.*, 2014). However, as both oligospermic and azoospermic men fall into this category, it is uncertain to what degree the azoospermic men might have influenced the risk estimates.

Azoospermia is the complete lack of sperm in the ejaculate and is estimated to affect 1% of the male population (Jarow *et al.*, 1989). Most cases are due to primary testicular failure (non-obstructive azoosper-

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mia), which may be linked to certain health conditions, such as Klinefelter syndrome, although the etiology is unknown in most cases (Cocuzza *et al.*, 2013). In fact, a previous report with UK and Danish data found an increased risk of death among men with Klinefelter syndrome (Bojesen and Gravholt, 2011). It is also well-known that a history of cancer and its treatment implies higher risk of infertility and might also lead to premature death. However, we did a sub-analysis excluding previous cancer cases, which implied a somewhat reduced HR, which, however, was still statistically significantly increased. This finding supports an association between male factor infertility and death, which was not explained by a previous cancer diagnosis. Nonetheless, azoospermic men constitute a vulnerable group with higher risks of Leydig cell malfunction and androgen deficiency. One Swedish study ($n = 206$) comparing fertile and infertile men found over a 10-fold increased risk of hypogonadism among azoospermic men (Bobjer *et al.*, 2012). In men, poor androgen production has been linked to adverse health outcomes including higher risks of metabolic syndrome (Laaksonen *et al.*, 2004), cardiovascular disease (Corona *et al.*, 2011), rheumatic autoimmune diseases (Baillargeon *et al.*, 2016) and overall mortality (Shores *et al.*, 2006). The mechanisms linking androgen deficiency to poor health are complex, but possibly—at least partly—driven by impairment of the anti-inflammatory effects exerted by testosterone, e.g. androgen deficiency might lead to insulin resistance and secretion of inflammatory markers, which leads to endothelial dysfunction and subsequent cardiovascular disease (Traish *et al.*, 2009). However, as some infertile men have compensated hypogonadism (Ventimiglia *et al.*, 2017) this hypothesis should be interpreted with some caution.

Male factor infertility may also have genetic causes. Up to 20% of azoospermic men have genetic anomalies, including deletions of the azoospermia factor region of the Y chromosome and structural chromosomal abnormalities (Lee *et al.*, 2011). In recent years, insights from comparative hybridization testing have revealed genomic instability, including copy number variations (especially deletions), among azoospermic men (Krausz and Riera-Escamilla, 2018, Yatsenko *et al.*, 2015). As a result, this susceptibility to DNA damage might affect not only spermatogenesis, but also the general health of these men, which is supported by the high percentage of infertile men who died of biological causes (Krausz and Riera-Escamilla, 2018). Furthermore, an extensive review found evidence that different infertility etiologies share particular genes and molecular pathways with a number of other pathologies, including various cancers (Tarin *et al.*, 2015). This is in line with our results as deaths from cancers were more prevalent among infertile men. In our study, incorporating deaths from external causes as a competing risk further increased the risk of death from other causes among infertile men, which supports the above hypothesis.

It is interesting to note that studies on the female side have shown that women in fertility treatment generally have lower death rates (Vassard *et al.*, 2018, Venn *et al.*, 2001). As the decision to have children is likely made at a time with no acute, life-threatening illness, it has been argued that a healthy selection of women into fertility treatment might explain these reduced death rates (Vassard *et al.*, 2018). Such conclusions do not seem to apply to the male counterparts. This is in line with results from a recent Swedish study of 459 766 men, which found higher prescription rates of medications related to metabolic syndrome among ICSI fathers compared with men who became fathers naturally (Elenkov *et al.*, 2018). Taken together with our results, this healthy patient effect does not seem to apply to the male population when the comparison group is restricted to include men who became fathers.

Strengths and limitations

The major strength of this study lies in the large sample size, the longitudinal design and inclusion of an age-matched comparison group of fathers who did not receive fertility treatment. Denmark has a long history of collecting information on births, deaths, emigrations and various socioeconomic data that enabled a strong statistical analysis (Thygesen *et al.*, 2011). Moreover, the sufficient number of men who died during follow-up enabled the possibility of sensitivity analyses on different types of male factor infertility and a descriptive analysis of causes of death.

Our study also has limitations. We relied on the assumption that men with recorded male factor infertility did indeed have poor semen quality although we acknowledge that semen parameters may vary from one sample to another. Given these variations, the oligospermic/normospermic men would be at increased risk of misclassification. This phenomenon may also partly explain why no significantly increased risk was reported among the oligospermic men. However, by inclusion of the non-MAR fathers, we expect to have reduced this limitation to some degree as these men were less likely to be infertile. However, it is important to note that some of these men may also have semen parameters below the normative reference level. In fact, the normative reference levels for semen quality have also changed during the study period and we lacked data on when the new guidelines were implemented in the fertility clinics. However, the differences in reference values are small for most parameters when considering both intra- and inter-clinic variations of semen quality (Jarow *et al.*, 2013) and will likely not have any major impact on the results. It should, however, be acknowledged that these results are based on semen analyses from all fertility clinics in Denmark, which means that inter-laboratory differences are likely (Jorgensen *et al.*, 1997). Given a true association between semen quality and death, such laboratory differences might attenuate the risk estimates.

Also, as our data was extracted from registers no information on health behavior, such as smoking habits and BMI, were available, which has also been the case in previous studies (Eisenberg *et al.*, 2014, Jensen *et al.*, 2009). A lack of adjustment for BMI and smoking could cause residual confounding if these factors are believed to be confounders. We have partly accounted for this problem by adjusting for educational attainment, which may be corroborated with health behavior (Brunello *et al.*, 2016), although we acknowledge that this adjustment cannot fully correspond to lifestyle factors, which would have been more favorable. However, it could be argued that smoking and BMI mediates, rather than confounds, the association as couples undergoing fertility treatment are inclined to improve their health. In that case no adjustments are needed as our aim was to estimate the total effect of male factor infertility on death. In keeping with this, it should be noted that educational attainment may also be associated with seeking fertility treatment according to US data (Anderson *et al.*, 2009). However, given that MAR treatment in Denmark is free under many circumstances in the public health care system, access to care is perhaps less of an issue in Denmark. Overall, 50% of all MAR

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treatments at the national level are provided in the public health care sector and treatment costs in the private sector are substantially lower in comparison to many other countries. Moreover, previous Danish data has shown that the occupational social class among couples undergoing fertility treatment in the public sector is very similar to the general population in the same age groups (Schmidt *et al.*, 2005). We also lacked information on whether the MAR cohort eventually became fathers. As fatherhood in itself has been linked to reduced death rates, it is uncertain to what degree this may have influenced our results (Ringback Weitoft *et al.*, 2004). However, as Danish data have shown that 71% of couples seeking fertility treatment become biological parents within 5 years (Malchau *et al.*, 2017) we believe the MAR cohort and external group were comparable, which is supported by the similarly distributed baseline characteristics. For the internal comparison, we excluded men with missing exposure data, which could have introduced selection bias. However, we compared their sociodemographic data with the remaining MAR cohort, which was similar. Also, as the men were relatively young at baseline, a longer follow-up time would have been ideal, and we cannot rule out that a longer follow-up time would have led to increased risk estimates among men with other types of infertility. In addition, as the MAR cohort includes couples who sought treatment for infertility, these results may have limited generalizability to infertile men who do not seek fertility treatment. Last, as the causes of deaths only were available for 29 deaths among azoospermic men, these results should be interpreted with caution.

Conclusion

Our nationwide cohort determined the risk of death among men that had undergone fertility treatment in Denmark. We observed an increased risk of death among azoospermic men while no increased risk was found with other types of infertility. For the men with azoospermia, further insight into causal pathways is needed to identify options for monitoring and prevention.

Authors' roles

All authors have contributed to the design of the study. C.H.G. analyzed data and drafted the manuscript. All authors contributed to data interpretation, critical revision of the paper and final approval of the manuscript.

Funding

This study is part of the ReproUnion collaborative study, co-financed by the European Union, Intereg V Öresund-Kattegat-Skagerrak (NYPS 20200407). C.G.'s research stay at Stanford was funded by grants from the University of Copenhagen, Kong Christian den Tiendes Fond, Torben og Alice Frimodt Fond and Julie Von Müllen Fond.

Conflict of interest

M.E. is an advisor for Sandstone and Dadi. None of the other authors report conflicts of interest.

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