Reproductive function in the sons of women who experienced stress due to bereavement before and during pregnancy: a nationwide population-based cohort study

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Objective: To estimate the association between prenatal exposure to maternal stress and reproductive disorders in Danish men, where prenatal stress exposure was defined as the mother's loss of a close relative during pregnancy or in the 12 months before conception.
Design: Population-based cohort study.
Setting: Not applicable.
Patient(s): All males born in Denmark between 1973 and 2008 (n = 1,217,576) and observed for up to 39 years.
Intervention(s): None.
Main Outcome Measure(s): Male reproductive function, defined using a composite outcome including congenital malformations of genital organs, testicular cancer, diagnosis of male infertility, or assisted conception use due to male factor infertility.
Result(s): In total, 28,986 men (2.4%) had been exposed to prenatal stress, and 62,929 (5.2%) experienced the composite outcome during the follow-up period. Prenatal exposure to stress was associated with an elevated risk of reproductive problems (hazard ratio [HR] 1.09; 95% CI, 1.04–1.15). The association was stronger when the exposure occurred during the first trimester of pregnancy, and for congenital malformations of genital organs. When focusing on infertility alone, we saw no evidence of increased risk (HR 0.90; 95% CI, 0.77–1.06). In addition, the probability of marrying a woman was lower for exposed men (HR 0.93; 95% CI, 0.89–0.98).
Conclusion(s): Prenatal stress in the form of the mother's bereavement during the first trimester of pregnancy is associated with a higher risk of reproductive disorders from congenital malformations of the genital organs in the male offspring. The lack of an association between maternal bereavement and later infertility in the exposed male offspring may be due in part to the men's lower probability of attempting to have children. (Fertil Steril® 2017;107:189–97. ©2016 by American Society for Reproductive Medicine.)
Key Words: Bereavement, cryptorchidism, hypospadias, pregnancy, stress, male infertility, reproductive function

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Denmark is among the countries with the highest use of medically assisted reproduction (1). Among children born in 2014, 8% were conceived using these techniques, half of them with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (2). Use of ICSI is often associated with poor sperm quality. It is estimated that a male factor, alone or in combination with a female factor, is present in 40% to 50% of couples who experience infertility (3).

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The etiology of male infertility remains largely unknown; certain congenital anomalies such as cryptorchidism and hypospadias, and some adult conditions such as poor sperm quality and testicular cancer, are considered symptoms of disrupted developmental of the reproductive system (4). Several prenatal exposures, mostly environmental, have been hypothesized to play a role in this syndrome (4, 5), but limited evidence exists for a role of maternal stress. In animal studies, maternal stress during pregnancy has been shown to affect fertility and reproductive behavior in the offspring (6–10). In humans, previous studies have suggested that men and women prenatally exposed to maternal bereavement had their first child slightly later and ended up with slightly fewer children (11, 12). Additionally, women born to mothers who had experienced bereavement during the first trimester of pregnancy were more likely than unexposed women to be diagnosed with infertility or to receive treatment (13).

In this study, we use several Danish population-based registries to examine the association between exposure to maternal bereavement after the death of a close relative before or during pregnancy and subsequent risk of reproductive disorders in sons, using a composite outcome of congenital malformations of genital organs, testicular cancer, and infertility.

MATERIALS AND METHODS

Our population-based cohort included 1,229,375 males born in Denmark between 1973 and 2008. Information was obtained from several Danish national registers, linked through the unique personal identification number assigned to all live-born children (Supplemental Table 1, available online). The Danish Civil Registration System (CRS) (14) contains information on all persons living in Denmark, including sex, date and place of birth, migration status, and identity of the parents, with continuously updated information on vital status, place of residence, and spouses, among other factors. This register permits the identification of all individuals and the linkage to their family members. To increase the likelihood of identifying close relatives of the mother in the registers, we restricted the study to men born to mothers of Danish origin. We excluded 11,799 men (1.0%) whose mothers could not be linked to any living relative during the relevant time period. The final study population consisted of 1,217,576 men born to 862,265 mothers (Supplemental Fig. 1, available online).

Exposure Definition

Men were considered exposed if their mother had lost an older child (including stillbirths), a parent, or a sibling during pregnancy or in the 12 months preceding conception. The exposure also included the death of a spouse/partner (the registered father of the index man) from the time of estimated conception through the end of pregnancy. The conception date was estimated by subtracting gestational age (GA) at birth (in days) from the date of birth. The GA was obtained from the Medical Birth Registry (MBR) (15) and was predominantly based on the date of the last menstrual period in the early years and on ultrasound in the later years. The GA was missing for all 180,090 boys born between 1973 and 1977, and in 89,100 (8.6%) of those born from 1978 and onward. For these individuals, the GA was assumed to be 39, 35, 32, and 31 weeks for singletons, twins, triplets, and quadruplets, respectively. These values were chosen as they have been used in previous studies (16); however, to assess whether this imputation had an impact on the estimates, we performed several sensitivity analyses, including only men with an observed GA and imputing different values for the missing GA, as previously described elsewhere (13).

Outcome Definition

We defined disorders of male reproductive function using a composite outcome, including congenital malformations of genital organs, testicular cancer, and diagnoses or treatment for male infertility. This information was obtained from two different registers: [1] the Danish National Hospital Register (NHR) (17), which includes all nonpsychiatric discharge diagnoses from hospitals since 1977 (from 1995, also outpatient diagnoses); and [2] the Danish In Vitro Fertilization Register (IVFR) (18), which was established in 1994 and covers all IVF/ICSI treatments with fresh and frozen embryos performed in public and private fertility clinics, regardless of whether they resulted in a pregnancy, but not ovulation induction or intrauterine insemination during the study period.

Diagnoses obtained from the NHR included male infertility, congenital malformations of genital organs (cryptorchidism, hypospadias, and other), and testicular cancer. Male infertility was also identified by a link to a female partner with a diagnosis of "female infertility associated with male factors." A woman was considered as the man's partner if she was married to him at the time of diagnosis or if she cohabited with him without being his mother, daughter, or sister. (In a sensitivity analysis, a man and a woman living together were not considered partners if there were other people living in the same address who were not close relatives.) We treated these relationships as time-varying in the analysis, starting on the day couples were married or moved in together and ending on the day they were divorced or moved apart (based on information obtained from the CRS).

In the IVFR, each treatment is identified by the woman's identification number, while the male's identification number is not always available. We considered a man to have an infertility diagnosis when a treatment cycle was performed because of male factor infertility, provided that the man had not had a previous vasectomy. When the man's identification number was missing, a man was considered as having an infertility problem if he was married or cohabitated with the identified woman at the time of treatment, based on the above definition.

Outcome "occurrence" was approximated by the date of any of the above diagnoses or treatments. We used the same approach for congenital malformations, even though they would have been present at birth. We performed a subanalysis starting the follow-up period at 18 years and focusing on infertility (thus ignoring malformations of genital organs and testicular cancer). This subanalysis was

restricted to men born before January 1, 1994, so that the youngest men would be at least 18 years old at the end of the follow-up period. The list of diagnoses and treatment codes is reported in Supplemental Table 2 (available online).

Statistical Analysis

The follow-up period started at birth and ended at the time of first appearance of any of the applicable conditions, death, emigration, or December 31, 2011, whichever came first. We used Cox proportional hazards models to estimate the association between exposure and age-specific rate of the composite outcome of male reproductive disorders. Men born between 1973 and 1976 entered the risk set on January 1, 1977, when the NHR became available; therefore, for these men, malformations of genital organs would only be known if they had been recorded several years after birth (we additionally performed sensitivity analyses excluding men born before 1977).

We considered exposure as binary (exposed or not to maternal bereavement) and further categorized the exposed into categories, depending on [1] the timing of the mother's relative's death with respect to conception/pregnancy: 7–12 months and 0–6 months before conception, first trimester (from last menstrual period to week 13 of gestation) and second/third trimesters (from week 14 to birth) (19); and [2] the type of death: "unexpected" (death with no identified causes, motor vehicle accident, suicide, and other accidental or violent causes) and death from other causes, based on the Danish Register of Causes of Death (20). We stratified the analysis depending on the relationship of the mother to the deceased (spouse/partner, older child, or parent/sibling). Each of these analyses was performed restricting to men whose mothers had an opportunity for exposure—that is, respectively, a link in the registers to a living spouse/partner at the time of conception (n = 1,191,085), at least one living older child or a stillbirth (n = 675,751), and a living parent or sibling (n = 785,307) in the year before conception.

All estimates were adjusted for parity of the mother (first, second, third, fourth, or more), date of birth of the man, and maternal age, both using restricted cubic splines with five knots. We used robust standard errors to account for the presence of men born from the same mother in the study population.

In subanalyses, we further adjusted the estimates for maternal socioeconomic status (income, highest education achieved, and cohabitation) at the time of birth, which was available from 1981 from the Danish Database for Labour Market Research (21), and restricted to men born at term (gestational age available from 1978 from the MBR). We also assessed whether the association with the exposure was consistent across each outcome: infertility, congenital malformations of genital organs, and testicular cancer (in a subanalysis, cryptorchidism was considered only when corrected by orchiopexy). For infertility, we evaluated whether the estimates changed when restricting the study population to men who were married or cohabiting with a woman (as a proxy for being in a stable relationship, assuming that these men would be more likely to be trying to have a child). Finally, we performed sensitivity analyses restricted to men with an observed GA and imputing different values for a missing GA.

All results are reported as hazard ratios (HR) with 95% confidence intervals (CI). The assumption of proportional rates was assessed using log-minus-log plots. All analyses were performed using STATA/SE 11 (Stata Corporation).

The study was approved by the Danish Data Protection Agency (nr. 2013–41–2569). All data are stored on a secure platform at Statistics Denmark.

RESULTS

Based on our definition, 28,986 men (2.4% of the study population) were exposed to prenatal stress after maternal bereavement. The proportion of exposed men increased over time because the number of missing links to relatives decreased as the registers became more complete with time. Exposed men were more frequently born preterm and with a lower birth weight, compared with unexposed men. Mothers of exposed men were older and had higher parity than those who were unexposed (partly due to the fact that mothers who lost an older child were older and had higher parity) (Table 1).

We excluded 32 exposed (0.1%) and 1,746 unexposed (0.1%) men whose follow-up period ended before January 1, 1977 (Supplemental Fig. 1, available online). Overall, 1,215,798 men were evaluated during a maximum time of 39 years (median time of 18.5 years). During the follow-up period, 62,929 (5.2%) men experienced the composite outcome of congenital malformations of genital organs, testicular cancer, or infertility.

Prenatal stress as defined in this study was associated with a 9% increased risk of the combined outcome (HR 1.09; 95% CI, 1.04–1.15). The association was stronger when exposure occurred during the first trimester of pregnancy (HR 1.24; 95% CI, 1.06–1.44). Results stratified by the type of relationship the mother had with the deceased are shown in Table 2. Further adjustment for maternal socioeconomic status and restricting to men born at term did not materially change the estimates (results not shown).

Subanalyses that considered congenital malformations of genital organs and testicular cancer separately are shown in Figure 1. Overall, there was an increased risk of cryptorchidism and hypospadias for men whose mothers were exposed during the first trimester of pregnancy. We saw similar estimates when stratifying by the relationship between the mother and the deceased (Supplemental Fig. 2, available online); as there were only 20 exposed cases of testicular cancer, this analysis was not performed. When focusing on infertility, we restricted the analysis to men born between 1973 and 1993, starting the follow-up period at age 18 (n = 651,038; Supplemental Fig. 3 and Supplemental Table 3, available online), and we saw no evidence of increased risk for the exposed men (Table 3).

Among men who married during the study period, 51.5% did so between 9 months before the birth of the child and 3 years after (32.3% married from 9 months before to when the child was 1 year old). Although the likelihood of cohabiting with a woman was similar in exposed and unexposed men, the probability of marrying was lower for exposed men (0.93; 95% CI, 0.89–0.98; Supplemental Table 4, available online). Estimates for infertility were similar in the subanalysis restricted

TABLE 1

Baseline characteristics of the maternal prenatal stress study population.

	Exposed (n = 28,986)		Unexposed (n = 1,188,590)	
Characteristics	**n**	**%**	**n**	**%**
Birth year				
1973–1977	1,227	4.2	178,863	15.0
1978–1982	2,399	8.3	152,768	12.9
1983–1987	3,841	13.3	145,380	12.2
1988–1992	5,417	18.7	168,641	14.2
1993–1997	5,792	20.0	179,116	15.1
1998–2002	5,099	17.6	168,804	14.2
2002–2008	5,211	18.0	195,018	16.4
Maternal age at birth of the index man (y)				
<20	584	2.0	40,941	3.4
20–24	4,989	17.2	258,670	21.8
25–29	10,108	34.9	445,657	37.5
30–34	8,939	30.8	316,296	26.6
35+	4,366	15.1	127,026	10.7
Maternal parity at birth of the index man				
First	9,556	33.0	528,896	44.5
Second	11,785	40.7	441,390	37.1
Third	5,535	19.1	160,667	13.5
Fourth or more	2,110	7.3	57,637	4.8
Preterm birth (<37 wk)[a]				
N	27,759		1,009,727	
Yes	2,071	7.5	57,288	5.7
No	24,820	89.4	864,207	85.6
Missing	868	3.1	88,232	8.7
Birth weight[b]				
N	27,423		976,710	
<2,500 g	1,638	6.0	44,837	4.6
2,500–3,250 g	6,615	24.1	215,261	22.0
3,250–4,000 g	13,257	48.3	468,295	47.9
>4,000 g	5,412	19.7	188,473	19.3
Missing	501	1.8	59,844	6.1
Maternal income[c]				
N	26,513		914,107	
No income	426	1.6	25,290	2.8
Low (1/3)	7,919	29.9	274,257	30.0
Middle (1/3)	9,170	34.6	284,676	31.1
High (1/3)	8,750	33.0	277,976	30.4
Missing	248	0.9	51,908	5.7
Maternal cohabitation[c]				
N	26,513		914,107	
Yes	13,256	50.0	445,165	48.7
No	13,009	49.1	417,041	45.6
Missing	248	0.9	51,901	5.7
Maternal highest education[c]				
N	26,513		914,107	
Lower secondary	8,375	31.6	243,011	26.6
Upper secondary	10,510	39.6	356,527	39.0
Higher education	5,825	22.0	194,461	21.3
Graduated studies	1,262	4.8	46,251	5.1
Missing	541	2.0	73,857	8.1

[a] Available in men born from 1978.
[b] Available in men born from 1979.
[c] Available in men born from 1981.

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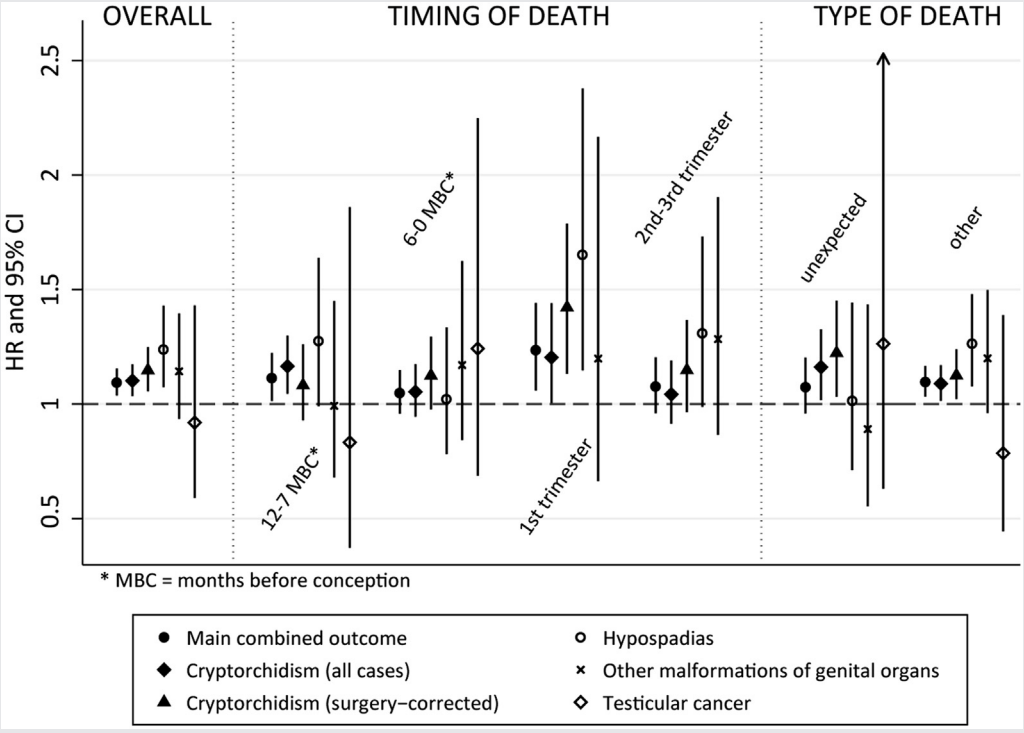
to men who were married or who cohabited with a woman, although in this population it was not uncommon to formalize a relationship after the conception (or birth) of a child (Supplemental Table 5, available online).

Sensitivity analyses excluding men born between 1973 and 1976 did not change the estimates. Further sensitivity analyses restricting to men with observed GA or imputing different values for missing a GA showed similar estimates, suggesting that the chosen imputed values did not impact the estimates (not shown). Finally, we saw similar estimates in the sensitivity analysis in which a man and a woman living together were not considered as partners if there

FIGURE 1



Hazard ratios and 95% confidence intervals for [1] main composite outcome of infertility, congenital malformations of genital organs, and testicular cancer; [2] cryptorchidism (all cases); [3] cryptorchidism (surgically corrected); [4] hypospadias; [5] other malformations of genital organs; and [6] testicular cancer according to exposure to prenatal stress after maternal bereavement. Estimates in strata with fewer than five cases are not shown.

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were other people living at the same address who were not close relatives.

DISCUSSION

In this study, we found that males exposed to prenatal stress after maternal bereavement had a slightly higher risk of reproductive disorders than unexposed males. The association was driven by an increased risk of congenital anomalies of genital organs. We observed no overall association with testicular cancer, or with diagnosis or treatment for infertility due to male factors.

It has been shown that stress hormones in pregnant women enter the fetal circulation (22) and that the fetus increases its own production of stress hormones (23, 24). Indeed, maternal anxiety is correlated with cortisol levels in the fetus (25), and such an increase may affect androgen production (26) and reproductive development.

In animals, the findings are inconsistent regarding whether maternal stress during pregnancy affects testosterone. Some studies suggest lower levels in exposed male rodents (6–8), but others do not (9). Nevertheless, male rodents exposed to maternal stress may have lower levels of luteinizing hormone and follicle-stimulating hormone (6, 7). Moreover, there is some evidence that exposure to prenatal stress is associated with reduced sexual activity, a decreased number of ejaculations (8–10), delayed testicular descent, and reduced anogenital distance (AGD) (7). The type of stress studied in most of these experimental studies is restraint or immobilization, which has been suggested to cause reactions similar to bereavement, such as anxiety and depression (27); however, animal models often suggest associations that are not replicated in humans (28).

Due to the difficulty of studying the long-term effects of prenatal exposures in humans, AGD has been used as a proxy of reproductive function in epidemiologic studies (29–31). Barrett et al. (32) showed that boys born to couples who experienced stress during pregnancy had slightly reduced AGD compared with those born to unexposed couples, although the differences were not statistically significant. In the same study, exposed daughters had longer AGD than unexposed daughters, suggesting that the effect of prenatal stress on reproductive function may be sex dependent, which is not unexpected given the very different physiology.

Previous studies have investigated the association between prenatal stress due to bereavement with hypospadias (33), cryptorchidism (34), and testicular cancer (35). Ingstrup et al. (33, 34) used the same source population and exposure as our study, with very similar results. However, the association between exposure to maternal bereavement and cryptorchidism was slightly stronger in our study, probably due to the more detailed adjustment for calendar time. For

TABLE 2

Hazard ratios and 95% confidence intervals for composite outcome of infertility, congenital malformations of genital organs, or testicular cancer according to exposure to prenatal stress after maternal bereavement depending on the relationship between the mother and the deceased.

				Hazard ratio			
Source of prenatal stress	**N**	**Years at risk**	**Events**	**Crude**	**Adjusted**	**95% CI**	
Death of any relative (spouse/partner, child, parent or sibling)							
Unexposed[b]	1,186,844	22,347,212	61,538	1.00	1.00	Reference	
Overall exposed	28,954	481,621	1,391	1.08	1.09	1.04	1.15
Exposed depending on timing of death							
12–7 mo before conception	8,999	152,284	445	1.09	1.11	1.01	1.22
6–0 mo before conception	10,099	174,327	479	1.03	1.05	0.96	1.15
1st trimester	3,149	49,335	164	1.24	1.24	1.06	1.44
2nd or 3rd trimester	6,707	105,674	303	1.08	1.08	0.96	1.20
Exposed depending on type of death[c]							
Sudden death	6,091	111,931	306	1.03	1.07	0.96	1.20
Other	22,568	364,976	1,069	1.09	1.10	1.03	1.17
Death of spouse/partner (n = 1,191,085)[a]							
Unexposed[b]	1,190,758	22,495,331	62,135	1.00	1.00	Reference	
Overall exposed	327	6,782	27	1.48	1.53	1.05	2.23
Exposed depending on timing of death							
12–7 mo before conception	—	—	—	—	—	—	—
6–0 mo before conception	—	—	—	—	—	—	—
1st trimester	66	1,307	4	NA	NA	NA	NA
2nd or 3rd trimester	261	5,475	23	1.57	1.63	1.08	2.44
Exposed depending on type of death[c]							
Sudden death	183	3,879	10	0.96	0.99	0.53	1.84
Other	132	2,655	14	1.95	2.00	1.18	3.39
Death of older child (n = 675,751)[a]							
Unexposed[b]	668,743	12,549,138	33,617	1.00	1.00	Reference	
Overall exposed	7,008	140,659	401	1.08	1.12	1.02	1.24
Exposed depending on timing of death							
12–7 mo before conception	2,832	56,661	149	1.00	1.03	0.87	1.21
6–0 mo before conception	3,911	78,614	234	1.13	1.17	1.03	1.34
1st trimester	133	2,742	12	1.67	1.74	1.00	3.02
2nd or 3rd trimester	132	2,641	6	0.89	0.92	0.41	2.08
Exposed depending on type of death[c]							
Sudden death	3,538	67,697	196	1.10	1.13	0.98	1.30
Other	3,426	72,251	201	1.06	1.10	0.96	1.27
Death of parent or sibling (n = 785,307)[a]							
Unexposed[b]	763,548	11,644,190	32,246	1.00	1.00	Reference	
Overall exposed	21,759	336,408	972	1.06	1.07	1.00	1.14
Exposed depending on timing of death							
12–7 mo before conception	6,150	95,429	296	1.14	1.16	1.03	1.30
6–0 mo before conception	6,239	96,498	251	0.95	0.96	0.85	1.09
1st trimester	2,978	45,668	147	1.17	1.18	1.00	1.39
2nd or 3rd trimester	6,392	98,813	278	1.03	1.04	0.93	1.18
Exposed depending on type of death[c]							
Sudden death	2,420	41,159	105	0.97	1.01	0.83	1.22
Other	19,095	291,422	858	1.07	1.08	1.01	1.16

Note: All estimates were obtained using Cox proportional hazards models adjusting with robust standard errors. Adjusted estimates were adjusted by maternal age, maternal parity, and date of birth. Estimates were not available (NA) in strata with fewer than five cases.

[a] Analyses were restricted to men born to mothers who had [1] a living spouse/partner at time of conception, at least [2] one living older child, and [3] a living parent or sibling in the year before pregnancy.

[b] The unexposed group was the reference group in all analyses.

[c] Type of death was missing for 295 men who lost any relative (16 cases), 12 men whose mother lost a spouse/partner (three cases), 44 men whose mother lost an older child (four cases), and 244 men whose mother lost a parent or sibling (nine cases).

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testicular cancer, Bermejo et al. (35) reported an increased risk among the exposed men, but their study was based on only 13 exposed cases. Our results were based on 20 exposed cases of testicular cancer, making estimates highly unstable and a comparison between the two studies difficult.

To the best of our knowledge, ours is the first study to examine the association between prenatal stress and male infertility. Although we saw a slightly increased risk of reproductive disorders based on the composite outcome, there was no association when considering the diagnosis of or treatment for infertility. We previously saw that men exposed to the same definition of maternal bereavement used in this study have fewer children and have them a little later than the unexposed men (11, 12). If these associations were causal, they could be due to a biological mechanism or result from differences in behavior. Given the data source, we have no way of knowing whether or when men wanted to have children; however, in this study, we saw that the exposed

TABLE 3

Hazard ratios and 95% confidence intervals for infertility according to exposure to prenatal stress after maternal bereavement depending on the relationship between the mother and the deceased, starting the follow-up at age 18 years.

				Hazard ratio			
	N	Years at risk	Events	Crude	Adjusted	95% CI	
Death of any relative (spouse/partner, child, parent or sibling)							
Unexposed[b]	637,734	6,307,738	15,749	1.00	1.00	Reference	
Overall exposed	13,304	94,522	151	0.95	0.90	0.77	1.06
Exposed depending on timing of death							
12–7 mo before conception	4,263	31,027	47	0.86	0.82	0.62	1.09
6–0 mo before conception	4,870	37,828	70	0.97	0.94	0.74	1.19
1st trimester	1,346	8,477	11	0.97	0.88	0.49	1.59
2nd or 3rd trimester	2,825	17,190	23	1.09	0.98	0.65	1.48
Exposed depending on type of death[c]							
Sudden death	3,336	27,017	44	0.82	0.79	0.59	1.06
Other	9,835	66,771	104	1.00	0.93	0.77	1.13
Death of spouse/partner (n = 644,100)[a]							
Unexposed[b]	643,891	6,350,179	15,828	1.00	1.00	Reference	
Overall exposed	209	2,101	7	1.36	1.39	0.67	2.92
Exposed depending on timing of death							
12–7 mo before conception	—	—	—	—	—	—	—
6–0 mo before conception	—	—	—	—	—	—	—
1st trimester	40	369	1	NA	NA	NA	NA
2nd or 3rd trimester	169	1,732	6	1.41	1.45	0.65	3.21
Exposed depending on type of death[c]							
Sudden death	121	1,219	1	NA	NA	NA	NA
Other	79	809	4	NA	NA	NA	NA
Death of older child (n = 358,823)[a]							
Unexposed[b]	354,590	3,511,821	8,807	1.00	1.00	Reference	
Overall exposed	4,233	41,011	97	0.98	0.99	0.81	1.21
Exposed depending on timing of death							
12–7 mo before conception	1,706	16,284	32	0.82	0.82	0.58	1.16
6–0 mo before conception	2,359	23,183	59	1.05	1.07	0.83	1.38
1st trimester	87	854	6	2.93	3.06	1.38	6.81
2nd or 3rd trimester	81	690	0	—	—	—	—
Exposed depending on type of death[c]							
Sudden death	2,042	18,655	36	0.83	0.84	0.60	1.16
Other	2,171	22,219	60	1.08	1.10	0.86	1.42
Death of parent or sibling (n = 311,261)[a]							
Unexposed[b]	302,339	1,849,175	2,428	1.00	1.00	Reference	
Overall exposed	8,922	51,732	47	0.77	0.77	0.58	1.03
Exposed depending on timing of death							
12–7 mo before conception	2,554	14,742	15	0.88	0.88	0.53	1.46
6–0 mo before conception	2,538	14,767	11	0.63	0.63	0.35	1.14
1st trimester	1,224	7,279	4	NA	NA	NA	NA
2nd or 3rd trimester	2,606	14,943	17	0.98	0.99	0.61	1.60
Exposed depending on type of death[c]							
Sudden death	1,193	7,249	7	0.79	0.79	0.37	1.66
Other	7,624	43,953	40	0.77	0.78	0.57	1.06

Note: All estimates were obtained using Cox proportional hazards models adjusting with robust standard errors. Adjusted estimates were adjusted by maternal age, maternal parity, and date of birth. Estimates were not available (NA) in strata with fewer than five cases.

[a] Analyses were restricted to men born to mothers who had [1] a living spouse/partner at time of conception, at least [2] one living older child, and [3] a living parent or sibling in the year before pregnancy.

[b] The unexposed group was the reference group in all analyses.

[c] Type of death was missing for 133 men who lost any relative (three cases), nine men whose mother lost a spouse/partner (two cases), 20 men whose mother lost an older child (one case), and 105 men whose mother lost a parent or sibling (no cases).

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men were slightly less likely to get married than the unexposed. Previous studies have suggested that prenatal stress may influence sexual orientation in men (36, 37). If exposed men were less likely to try to have children, or started later, they would seek help for infertility less frequently or later, which may explain in part the absence of an association, given the incomplete follow-up period. When we restricted the analysis to men who were married or who cohabited with a woman, we found no differences. However, a substantial proportion of men formalized their relationship around the time of birth of their first child; as a result, restricting the analyses to men in a relationship would not necessarily increase the likelihood of identifying men trying to have children.

The main strength of this study is the use of virtually complete population registries, which results in minimizing the potential for selection and information bias. The death of a close relative is one of the most stressful life events

(38), making our exposure a reasonable proxy of stress that would result in a marked exposure contrast. Indeed, previous studies have shown the effects of losing a relative on health and life expectancy, both in bereaved parents (39, 40) and in children born after being exposed in utero (41, 42).

There are also several limitations in studies based on administrative registers. The timing of exposure was based on the date of death of the family member, but stress could, at least in some instances, have been present from an earlier time, and we cannot measure its duration or intensity. A potential source of misclassification of the exposure is the incomplete linkage of mothers to their relatives, a phenomenon that mostly occurred in the early years of the registers because mothers not sharing an address with their parents when the CRS started in 1968 had no links to them and, as a result, to their siblings. For this reason, we carefully adjusted all estimates by birth year and restricted the analyses to individuals with a linkage to the relevant family members, who were thus at risk of being exposed. Nevertheless, it is likely that such misclassification of the exposure would be nondifferential, biasing the estimates toward the null.

Reproductive function was also defined indirectly, using a composite outcome based on registers with different starting times. The NHR started in 1977, preventing us from identifying congenital malformations diagnosed at birth in boys born between 1973 and 1976. However, sensitivity analyses restricting the population to men born after January 1, 1977, showed similar results to those observed in the complete analysis. Cryptorchidism and hypospadias are assessed in all boys at birth and reported to the NHR. Thus, the register includes cases of boys with undescended testis at birth that later descended spontaneously. A subanalysis including only cases of cryptorchidism corrected by surgery showed slightly stronger associations between first-trimester prenatal exposure to bereavement and cryptorchidism. The NHR used the International Classification of Diseases, 8th version (ICD-8) until the end of 1993 and the 10th version (ICD-10) thereafter. The diagnosis of "female infertility associated with male factors" was only present in the ICD-10; it was thus possible to assess male infertility through the female partner only from January 1, 1994, the same date that the IVFR started. However, at that time, the men in the earliest cohort—born in 1973—were 21 years old, and we believe that only a small minority of men would have sought help for infertility at an earlier age. The fact that the oldest men in the cohort were 39 years old at the end of the follow-up period is another important limitation because many men would not have completed their reproductive life by that age, while a large proportion were too young by the end of follow-up period to be diagnosed with infertility. This study might thus be underpowered to detect an effect of maternal stress on sons' infertility.

Our results suggest that men exposed to maternal bereavement after the death of a close relative during pregnancy or in the year before conception are more likely to experience reproductive disorders, mostly due to a higher risk of having been born with congenital malformations of genital organs. The association was stronger when exposure occurred during the first trimester of pregnancy. The lack of association between prenatal stress and subsequent male infertility could be in part a result of exposed men being less likely to try to have children than the unexposed, and therefore less likely to seek help for infertility, or seek it later.

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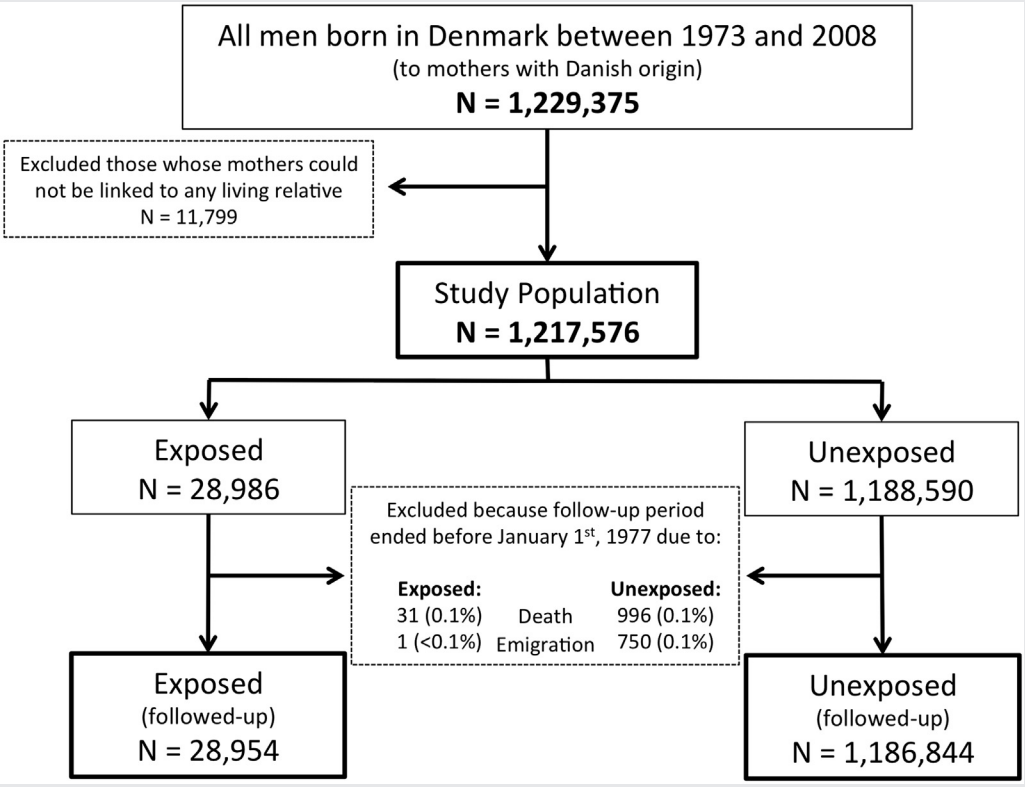
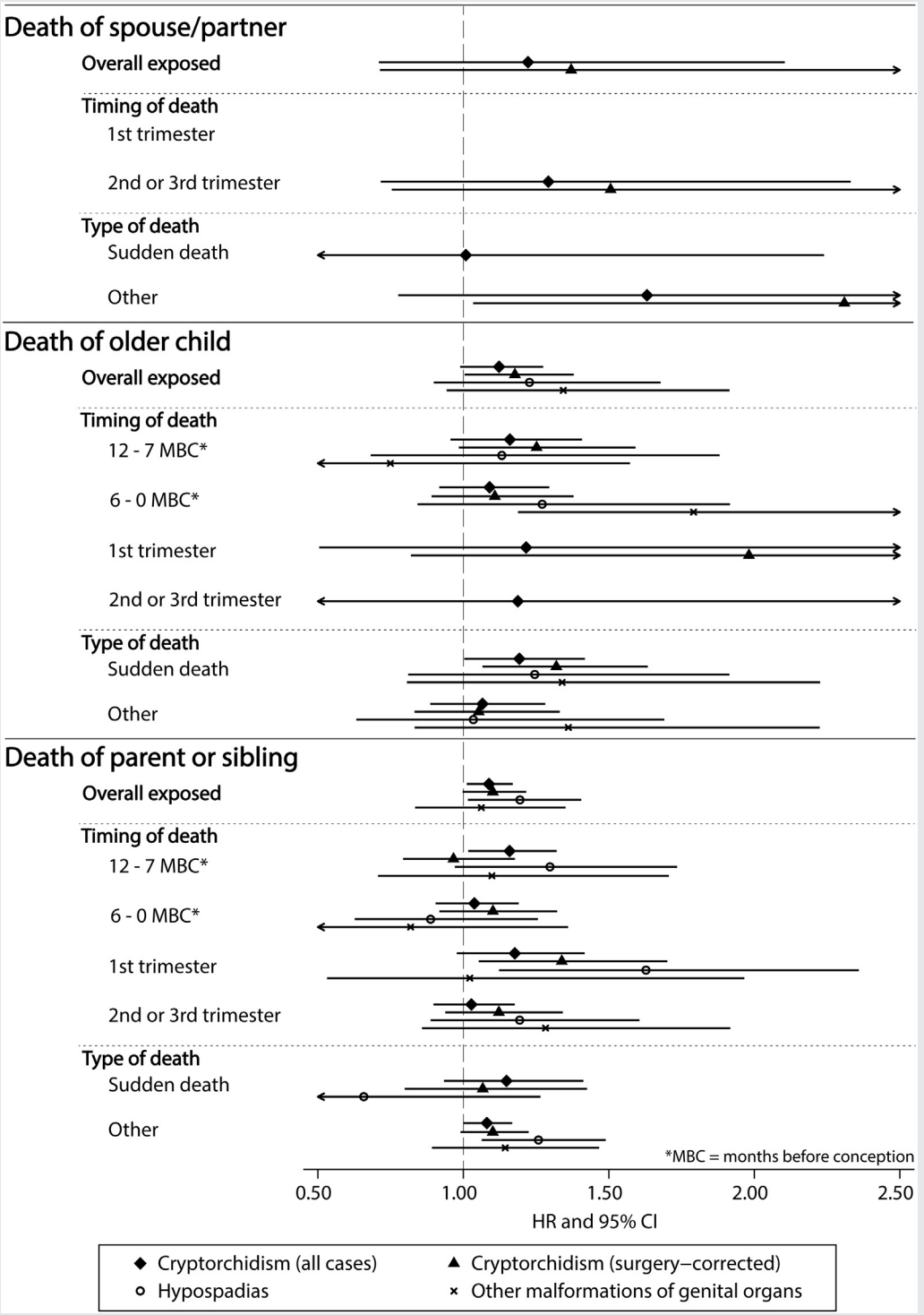
SUPPLEMENTAL FIGURE 1



Formation of the study population.

Plana-Ripoll. Prenatal stress and male reproduction. Fertil Steril 2016.

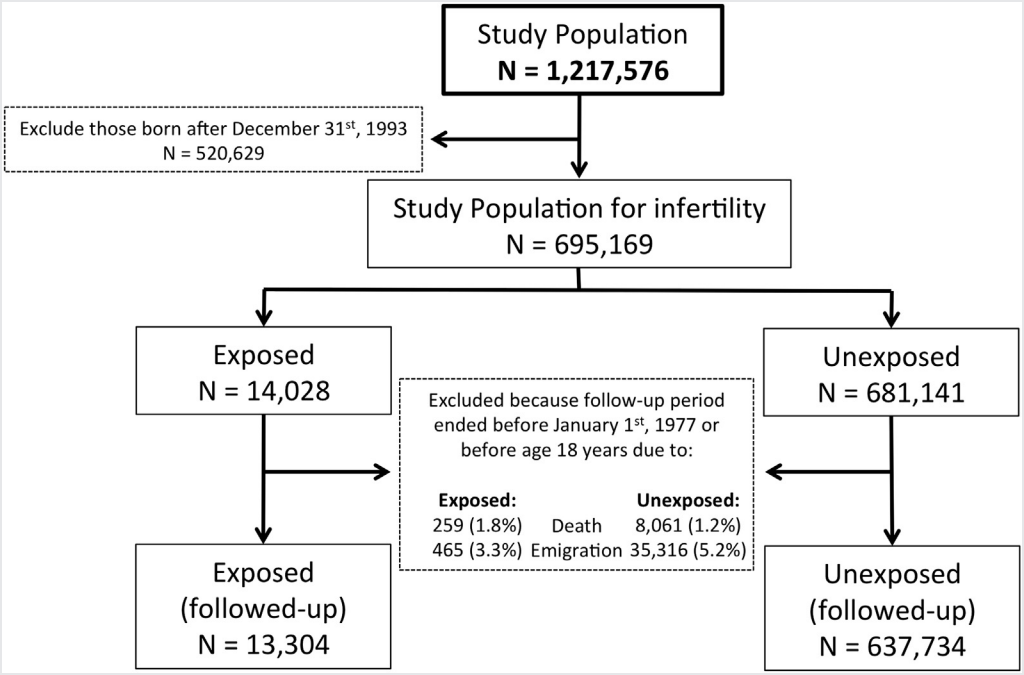
SUPPLEMENTAL FIGURE 2



Hazard ratios and 95% confidence intervals for [1] cryptorchidism (all cases); [2] cryptorchidism (surgically corrected); [3] hypospadias; and [4] other malformations of genital organs according to exposure to prenatal stress after maternal bereavement depending on the relationship between the mother and the deceased. Estimates in strata with fewer than five cases are not shown.

Plana-Ripoll. Prenatal stress and male reproduction. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 3



Formation of the study population for the subanalysis considering infertility (excluding congenital malformations of genital organs and testicular cancer).

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SUPPLEMENTAL TABLE 1

Summary of information used from each of the national registers.

Register	Information used
Civil Registration System	Date of birth, vital status, emigration, place of residence, spouses, and linkage to family members
Medical Birth Registry	Date of birth, gestational age, maternal parity
Register of Causes of Death	Relatives' date and cause of death
National Patient Register	Date of admission/ outpatient appointment and diagnoses
Integrated Database for Labour Market Research	Maternal education, income, and cohabitation
In Vitro Fertilization Register	Fertility treatment and underlying causes of infertility

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SUPPLEMENTAL TABLE 2

Definition of outcomes of infertility.

Identification through index men in the study population

Danish National Hospital Register (available from January 1, 1977)		
Diagnoses	ICD-8 code	ICD-10 code
Male infertility	606	N46
Congenital malformations[a]		
Cryptorchidism[b]	752.1	Q53
Hypospadias	752.2	Q54 (except Q54.4)
Other	752.0, 752.7, 752.8, 752.9	Q54.4, Q55, Q56
Testicular cancer[a]	186	C62

Identification through female partner of men in the study population

Danish National Hospital Register (available from January 1, 1977)		
Diagnoses	ICD-8 code	ICD-10 code
Female infertility associated with male factors	—	N97.4
Danish IVF Register (available from January 1, 1994)		
Treatment	Considerations	
Female infertility (male cause[c])	All observations in the register in which there is a male cause[c] and the man did not have a previous vasectomy (codes KKFD46 and KKFD50 in the Nordic Classification of Surgical Procedures)	

Note: The Danish National Hospital Register used the *International Classification of Diseases*, 8th version (ICD-8) until the end of 1993 and the 10th version (ICD-10) thereafter. IVF = in vitro fertilization.

[a] Congenital malformations and testicular cancer were excluded from a subanalysis starting the follow-up period at age 18 years.

[b] In a subanalysis, cryptorchidism was considered only when corrected by orchiopexy (codes 55600, 55640, KKFH00, KKFH01 and KKFH10 in the Nordic Classification of Surgical Procedures).

[c] The IVF Register includes a variable for "history of male abnormalities" in the period 1994–2005, which was used to identify male factor infertility. In the period 2006–2011, a diagnosis for male and female causes is registered using ICD-10 codes. We considered that male factor infertility was present when the female cause was listed as "female infertility associated with male factors" (N97.4) and/or the diagnosis for the male was "male infertility" (N46).

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SUPPLEMENTAL TABLE 3

Baseline characteristics of study population born before January 1, 1994.

	Exposed (n = 14,028)		Unexposed (n = 681,141)	
Characteristics	**N**	**%**	**N**	**%**
Birth year				
1973–1977	1,227	8.7	178,863	26.3
1978–1982	2,399	17.1	152,768	22.4
1983–1987	3,841	27.4	145,380	21.3
1988–1993	6,561	46.8	204,130	30.0
Maternal age in years at birth of the index man				
<20	427	3.0	32,314	4.7
20–24	3,629	25.9	193,482	28.4
25–29	5,796	41.3	265,594	39.0
30–34	3,315	23.6	140,974	20.7
35+	861	6.1	48,777	7.2
Maternal parity at birth of the index man				
First	4,854	34.6	306,924	45.1
Second	5,720	40.8	253,205	37.2
Third	2,564	18.3	89,249	13.1
Fourth or more	890	6.3	31,763	4.7
Preterm birth (<37 wk)[a]				
N	12,801		502,278	
Yes	849	6.6	24,263	4.8
No	11,350	88.7	412,307	82.1
Missing	602	4.7	65,708	13.1
Birth weight[b]				
N	12,465		469,261	
<2,500 g	755	6.1	21,667	4.6
2,500–3,250 g	3,243	26.0	110,811	23.6
3,250–4,000 g	6,124	49.1	222,604	47.4
>4,000 g	2,157	17.3	77,815	16.6
Missing	186	1.5	36,364	7.7
Maternal income[c]				
N	11,555		406,658	
No income	331	2.9	16,630	4.1
Low (1/3)	3,574	30.9	118,044	29.0
Middle (1/3)	3,916	33.9	121,736	29.9
High (1/3)	3,620	31.3	118,754	29.2
Missing	114	1.0	31,494	7.7
Maternal cohabitation[c]				
N	11,555		406,658	
Yes	5,799	50.2	198,880	48.9
No	5,642	48.8	176,284	43.3
Missing	114	1.0	31,494	7.7
Maternal highest education[c]				
N	11,555		406,658	
Lower secondary	4,676	40.5	138,764	34.1
Upper secondary	4,199	36.3	141,953	34.9
Higher education	2,101	18.2	71,784	17.7
Graduated studies	279	2.4	10,372	2.6
Missing	300	2.6	43,785	10.8

[a] Available in men born from 1978.
[b] Available in men born from 1979.
[c] Available in men born from 1981.

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SUPPLEMENTAL TABLE 4

Exposed and unexposed men being married to a woman before December 31, 2014, age at first marriage, and duration of marriage depending on birth year.

	Men born in 1973–1977				Men born in 1978–1982				Men born in 1983–1987				Men born in 1988–1992			
	Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed		Exposed		Unexposed	
Relationship parameters	**1,227**		**178,863**		**2,399**		**152,768**		**3,841**		**145,380**		**5,417**		**168,641**	
Cohabited with a woman	1,113	90.7%	165,086	92.3%	2,141	89.2%	136,959	89.7%	3,327	86.6%	123,858	85.2%	3,609	66.6%	112,733	66.8%
Age at first cohabitation	22.1	20.0–24.6	22.1	20.2–24.5	22.0	20.1–24.4	22.1	20.3–24.5	21.8	20.2–23.9	21.9	20.2–24.1	21.2	19.8–22.4	21.1	19.7–22.5
Duration of first cohabitation	3.0	1.2–8.0	2.7	1.0–7.8	2.5	1.0–7.1	2.4	1.0–6.9	2.3	1.0–5.7	2.2	0.9–5.3	1.9	0.9–3.3	1.7	0.9–3.3
Married to a woman	674	54.9%	103,230	57.7%	946	39.4%	63,789	41.8%	637	16.6%	26,570	18.3%	140	2.6%	5,084	3.0%
Age at first marriage	30.1	28.0–33.1	30.2	27.7–33.4	29.5	27.0–31.4	29.3	27.0–31.2	27.2	25.3–28.6	27.0	25.0–28.7	23.5	22.4–24.6	23.5	22.1–24.9
Duration of first marriage	8.3	5.4–11.2	8.6	5.5–11.7	5.4	3.3–7.4	5.7	3.5–8.3	3.4	2.1–4.7	3.4	2.2–5.4	2.2	1.5–3.3	2.4	1.5–3.6

Note: Information on age and duration is provided in years by the median and the interquartile range.

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SUPPLEMENTAL TABLE 5

Hazard ratios and 95% confidence intervals for infertility according to exposure to prenatal stress after maternal bereavement depending on the relationship between the mother and the deceased, starting the follow-up period at age 18 years and restricted to men in a relationship with a female partner.

				Hazard ratio			
Bereavement parameter	**N**	**Years at risk**	**Events**	**Crude**	**Adjusted**	**95% CI**	
Death of any relative (spouse/partner, child, parent, or sibling)							
Unexposed[b]	447,415	2,405,979.1	13,643	1.00	1.00	Reference	
Overall exposed	7,924	30,942.2	126	0.91	0.88	0.74	1.05
Exposed depending on timing of death							
12–7 mo before conception	2,548	10,311.3	38	0.80	0.77	0.56	1.06
6–0 mo before conception	3,043	12,686.8	59	0.97	0.95	0.73	1.22
1st trimester	766	2,738.6	10	0.96	0.88	0.47	1.63
2nd or 3rd trimester	1,567	5,202.5	19	1.02	0.92	0.59	1.45
Exposed depending on type of death[c]							
Sudden death	2,171	9,668.9	37	0.78	0.77	0.56	1.06
Other	5,688	21,062.6	86	0.96	0.91	0.73	1.12
Death of spouse/partner (n = 451,097)[a]							
Unexposed[b]	450,942	2,420,870.1	13,713	1.00	1.00	Reference	
Overall exposed	155	881.7	6	1.25	1.30	0.59	2.89
Exposed depending on timing of death							
12–7 mo before conception	—	—	—	—	—	—	—
6–0 mo before conception	—	—	—	—	—	—	—
1st trimester	27	181.7	1	NA	NA	NA	NA
2nd or 3rd trimester	128	700.0	5	1.28	1.32	0.55	3.17
Exposed depending on type of death[c]							
Sudden death	86	533.9	1	NA	NA	NA	NA
Other	63	323.8	3	NA	NA	NA	NA
Death of older child (n = 251,775)[a]							
Unexposed[b]	248,779	1,342,043.9	7,594	1.00	1.00	Reference	
Overall exposed	2,996	15,451.4	81	0.95	0.97	0.78	1.21
Exposed depending on timing of death							
12–7 mo before conception	1,196	6,194.9	27	0.79	0.80	0.55	1.17
6–0 mo before conception	1,685	8,631.1	49	1.02	1.06	0.80	1.40
1st trimester	66	358.0	5	2.66	2.79	1.13	6.90
2nd or 3rd trimester	49	267.4	0	—	—	—	—
Exposed depending on type of death[c]							
Sudden death	1,404	6,997.2	31	0.82	0.83	0.58	1.18
Other	1,579	8,391.8	49	1.03	1.07	0.81	1.42
Death of parent or sibling (n = 172,755)[a]							
Unexposed[b]	167,949	547,800.1	2,108	1.00	1.00	Reference	
Overall exposed	4,806	14,713.6	39	0.73	0.74	0.54	1.01
Exposed depending on timing of death							
12–7 mo before conception	1,350	4,112.5	11	0.74	0.75	0.41	1.35
6–0 mo before conception	1,374	4,076.0	10	0.68	0.68	0.37	1.27
1st trimester	676	2,214.9	4	NA	NA	NA	NA
2nd or 3rd trimester	1,406	4,310.2	14	0.92	0.93	0.55	1.58
Exposed depending on type of death[c]							
Sudden death	692	2,168.8	5	0.66	0.66	0.27	1.59
Other	4,067	12,417.3	34	0.75	0.75	0.54	1.06

Note: All estimates were obtained using Cox proportional hazards models adjusting with robust standard errors. Adjusted estimates were adjusted by maternal age, maternal parity and date of birth. Estimates were not available (NA) in strata with less than five cases.

[a] Analyses were restricted to men born to mothers who had [1] a living spouse/partner at time of conception, at least [2] one living older child, and [3] a living parent or sibling in the year before pregnancy.

[b] The unexposed group was the reference group in all analyses.

[c] Type of death was missing for 133 men who lost any relative (three cases), nine men whose mother lost a spouse/partner (two cases), 20 men whose mother lost an older child (one case), and 105 men whose mother lost a parent or sibling (no cases).

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