Supplemental material

Supplemental material 1

Selection weights were applied to account for the potential risk of selection bias due to non-participation.(Hernan, Hernandez-Diaz et al. 2004) Baseline information from the first questionnaire in the DNBC was an eligibility criterion for the FEPOS cohort, and therefore almost complete information on baseline characteristics for both participants and non-participants were available. Using this information, it was possible to make selection weights to reweight the population to a pseudo population representative of all invited for the FEPOS cohort.

The selection weight for the pregnancy exposure analyses were estimated using a logistic regression model on participation status (yes, no) with the primary exposure variable (parental separation in pregnancy: yes, no) and selected covariates potentially associated with participation as explanatory variables. These covariates included maternal socioeconomic status, maternal first trimester smoking, and maternal age at delivery, in addition to covariates potential associated with childhood hardship and stress: maternal alcohol consumption in the first trimester (yes, no); time-to-pregnancy (TTP) (unplanned, TTP of 0-12 months, TTP of >12 months or medically assisted reproduction); maternal work status in the first trimester (yes, no); and maternal house ownership (yes, shared ownership, rental, no housing). Moreover, region of invitation of the men (Aarhus or Copenhagen) was included to account for potential differences between clinics and geographic uptake.

For parental separation in pregnancy, 1,025 participants with full information on all covariates (complete cases) had a mean weight of 5.1 (range 3.1 – 13.9), which is equivalent to a pseudo population of 5,545 participants. The inverse of this weight was applied all pregnancy exposure analyses, making the associations count for almost all invited participants.

For parental separation in childhood, two different inverse probability weights were calculated, since the information on the exposure was obtained in a later follow-up wave at 11 years with non-participation of 45%, giving 2,964 participants in the 11-year follow-up from all invited men (5,697). Therefore, we first calculated a weight for participation in FEPOS among all men participating in the 11-year follow-up wave and next we calculated a weight for participation in the 11-years follow-up wave among all invited sons. This way, the 696 participants with complete information were re-

weighted up to the 2,964 participants in the 11-years follow-up, and next the 2,964 participants were re-weighted up to the 5,697 invited to FEPOS.

The first weight was calculated using a logistic regression model on participation status in FEPOS (yes, no) with the primary exposure variable (parental separation in childhood: yes, no) and the same selected covariates potentially associated with participation as described above, as explanatory variables. The first weight was used to re-weight the 696 participants with complete information from FEPOS up to a pseudo population of 2,969 participants from the 11-year follow-up wave, with a mean weight of 4.4 (range 1.9–9.7). The second weight was estimated using a logistic regression model on participation status in the 11-year follow-up wave (yes, no) with the same selected covariates potentially associated with participation as described above, as explanatory variables. In the second weight, the 2,964 participants from the 11-year follow-up were re-weighted up to a pseudo population of 5,537 participants (mean weight 1.9 (range 1.4–3.8)). The two weights were multiplied (joint mean weight 8.2 (range (3.7–23.2)) and the inverse of the weights were employed all analyses examining parental separation in childhood (both dichotomized and categorized), making the results applicable for all invited sons. Robust standard errors were used to account for the use of selection weights.

Reference

Hernan, M. A., S. Hernandez-Diaz and J. M. Robins (2004). "A structural approach to selection bias." Epidemiology **15**(5): 615-625.

Table S1.

Main results for parental separation in pregnancy or in childhood.
Crude and adjusted[a] (95% confidence intervals) relative percentage differences in semen quality according to parental separation in pregnancy or in childhood. Parental separation relatively to no parental separation

	Parental separation in pregnancy			Parental separation in childhood		
	n[b]	Crude	Adjusted (95% CI)	n[b]	Crude	Adjusted (95% CI)
Semen quality						
Volume (ml)[c]	834	5%	13 % (-6;36)	537	-7%	-7% (-16;2)
Concentration (mill/ml)	1007	-16%	-13% (-42;30)	656	-12%	-12% (-25;4)
Total sperm count (mill)[c]	834	-4%	12% (-28;76)	537	-12%	-10% (-25;8)
Motility (modelled as NP+IM %)[d]	984	-4%	-7% (-19;8)	643	-4%	-1% (-9;7)
Morphology (% normal)	984	16%	12% (-18;52)	642	0%	0% (-13;14)
Testes volume (ml)	1017	8%	9% (-6;26)	667	-3%	-2% (-9;5)

[a] Adjusted for maternal age at delivery; maternal social class; maternal first trimester smoking; abstinence time; place of semen sample collection (not testes volume); spillage (not testes volume).

[b] The numbers are from the adjusted model and vary due to exclusion due to azoospermia or potential missing information on covariates.

[c] Excluding samples with spillage

[d] Further adjusted for interval between ejaculation and analysis in minutes. Due to model fit, tables show results for NP (Non-progressive) + IM (Immotile) spermatozoa. Therefore, negative estimates represent a higher proportion of progressive motility, and positive estimates represent a lower proportion of progressive motility.

Table S2.

Main results exploring the timing of parental separation in childhood. Crude and adjusted[a] (95% confidence intervals) relative percentage differences in semen quality according to timing of parental separation in childhood. Parental separation from birth, in early childhood or in late childhood relatively to no parental separation

| | n[b] | Parental separation from birth | | Parental separation in early childhood | | Parental separation in late childhood | |
		Crude	Adjusted (95% CI)	Crude	Adjusted (95% CI)	Crude	Adjusted (95% CI)
Semen quality							
Volume (ml)[c]	537	22%	25% (-5;64)	-12%	-14% (-24;-3)	-6%	-3% (-16;12)
Concentration (mill/ml)	656	0%	16% (-30;91)	-17%	-15% (-28;1)	-8%	-11% (-33;19)
Total sperm count (mill)[c]	537	22%	62% (-6;179)	-19%	-17% (-32;2)	-8%	-9% (-32;21)
Motility (modelled as NP+IM %)[d]	643	0%	-1% (-15;16)	-2%	4% (-8;17)	-7%	-7% (-17;5)
Morphology (% normal)	642	53%	59% (20;111)	-4%	-4% (-20;16)	-3%	-3% (-21;19)
Testes volume (ml)	667	14%	10% (-12;37)	-11%	-11% (-18;-3)	4%	6% (-3;17)

[a] Adjusted for maternal age at delivery; maternal social class; maternal 1first trimester smoking; abstinence time; place of semen sample collection (not testes volume); spillage (not testes volume).

[b] The numbers are from the adjusted model and vary due to exclusion due to azoospermia or potential missing information on covariates. In total, n = 10 was exposed to parental separation from birth in all analyses; n = 63-77 was exposed to parental separation from early childhood; and n = 53-63 was exposed to parental separation from late childhood.

[c] Excluding samples with spillage

[d] Further adjusted for interval between ejaculation and analysis in minutes. Due to model fit, tables show results for NP (Non-progressive) + IM (Immotile) spermatozoa. Therefore, negative estimates represent a higher proportion of progressive motility, and positive estimates represent a lower proportion of progressive motility.

Table S3.

Sub analysis further adjusting for planned or unplanned pregnancy.
Crude and adjusted[a] (95% confidence intervals) relative percentage differences in semen quality according to parental separation in pregnancy or in childhood. Parental separation relatively to no parental separation

	n[b]	Crude	Adjusted (95% CI)	n[b]	Crude	Adjusted (95% CI)
		Parental separation in pregnancy			Parental separation in childhood	
Semen quality						
Volume (ml)[c]	834	5%	6 % (-13;29)	537	-7%	-8% (-17;2)
Concentration (mill/ml)	1007	-16%	-10% (-41;37)	656	-12%	-11% (-25;5)
Total sperm count (mill)[c]	834	-4%	12% (-30;80)	537	-12%	-8% (-29;9)
Motility (modelled as NP+IM %)[d]	984	-4%	-5% (-18;10)	643	-4%	-1% (-9;8)
Morphology (% normal)	984	16%	15% (-16;58)	642	0%	0% (-12;16)
Testes volume (ml)	1017	8%	9% (-6;26)	667	-3%	-2% (-9;5)

[a] Adjusted for maternal age at delivery; maternal social class; maternal first trimester smoking; abstinence time; place of semen sample collection (not testes volume); spillage (not testes volume) in addition to planned or unplanned pregnancy.

[b] The numbers are from the adjusted model and vary due to exclusion due to azoospermia or potential missing information on covariates.

[c] Excluding samples with spillage

[d] Further adjusted for interval between ejaculation and analysis in minutes. Due to model fit, tables show results for NP (Non-progressive) + IM (Immotile) spermatozoa. Therefore, negative estimates represent a higher proportion of progressive motility, and positive estimates represent a lower proportion of progressive motility.

Table S4.

Sub analysis further adjusting for planned or unplanned pregnancy.
Crude and adjusted[a] (95% confidence intervals) relative percentage differences in semen quality according to timing of parental separation in childhood. Parental separation from birth, in early childhood or in late childhood relatively to no parental separation

	n[b]	Parental separation from birth		Parental separation in early childhood		Parental separation in late childhood	
		Crude	Adjusted (95% CI)	Crude	Adjusted (95% CI)	Crude	Adjusted (95% CI)
Semen quality							
Volume (ml)[c]	537	22%	21% (-9;63)	-12%	-14% (-25;-4)	-6%	-3% (-16;12)
Concentration (mill/ml)	656	0%	26% (-24;110)	-17%	-14% (-28;2)	-8%	-10% (-33;19)
Total sperm count (mill)[c]	537	22%	72% (1;193)	-19%	-17% (-32;3)	-8%	-9% (-32;22)
Motility (modelled as NP+IM %)[d]	643	0%	1% (-14;18)	-2%	4% (-7;17)	-7%	-6% (-17;6)
Morphology (% normal)	642	53%	77% (29;144)	-4%	-2% (-19;18)	-3%	-2% (-20;20)
Testes volume (ml)	667	14%	10% (-12;38)	-11%	-11% (-18;-2)	4%	6% (-4;17)

[a] Adjusted for maternal age at delivery; maternal social class; maternal first trimester smoking; abstinence time; place of semen sample collection (not testes volume); spillage (not testes volume) in addition to planned or unplanned pregnancy.

[b] The numbers are from the adjusted model and vary due to exclusion due to azoospermia or potential missing information on covariates. In total, n = 10 was exposed to parental separation from birth in all analyses; n = 63-77 was exposed to parental separation in early childhood; and n = 53-63 was exposed to parental separation in late childhood.

[c] Excluding samples with spillage

[d] Further adjusted for interval between ejaculation and analysis in minutes. Due to model fit, tables show results for NP (Non-progressive) + IM (Immotile) spermatozoa. Therefore, negative estimates represent a higher proportion of progressive motility, and positive estimates represent a lower proportion of progressive motility.