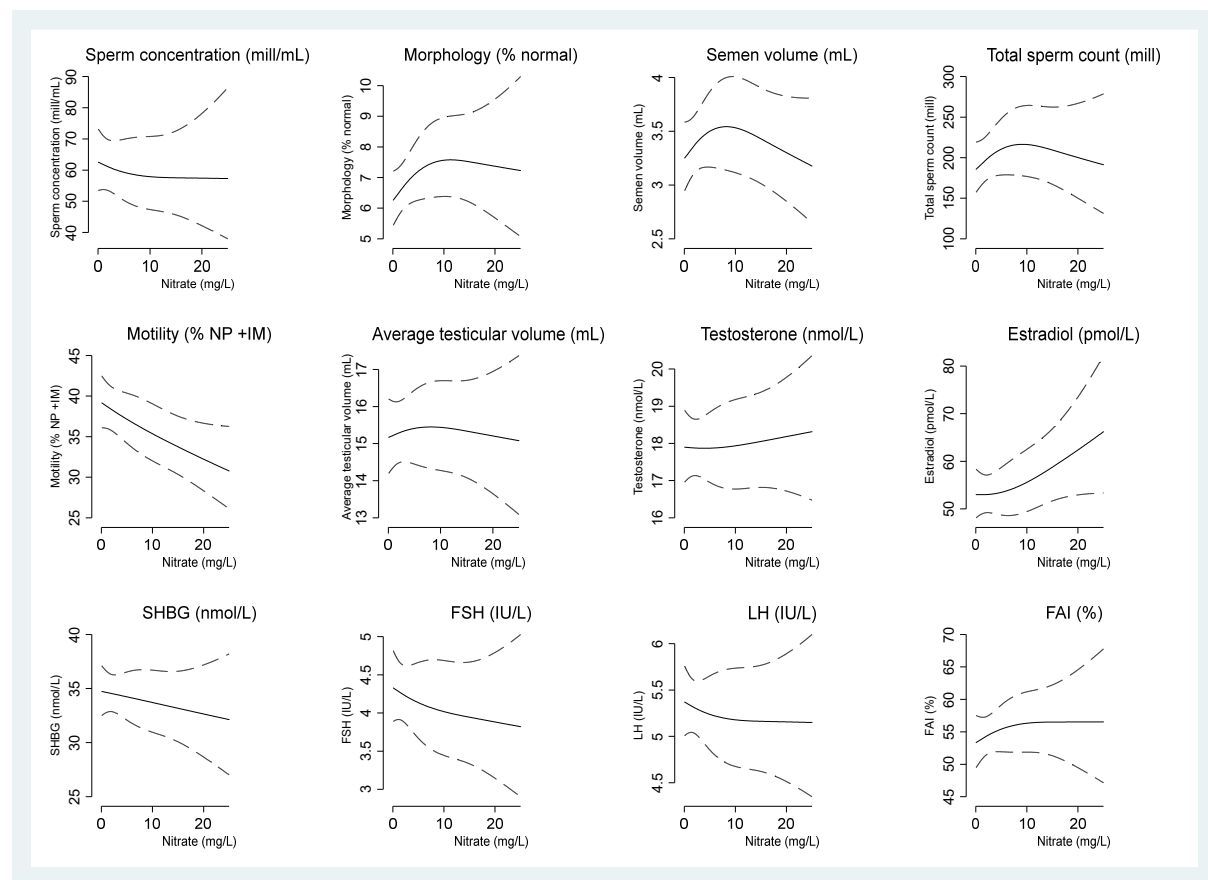


## Supplementary Materials



**Figure S1.** Spline plots with three knots (10th, 50th, 90th percentiles) and 95% confidence intervals showing the association between prenatal exposure to nitrate and semen characteristics, testes volume and reproductive hormones.

*The spline plots are made for a reference son who had an abstinence time of 2-3 days, delivered the semen sample at the clinic, reported no spillage, had the semen sample analyzed <60 minutes from delivery and had the blood test in the afternoon. The reference sons' mother were a non-smoker during pregnancy and aged 30 years at delivery. The highest educational level of the reference sons' parents were high-grade professional.*

**Table S1.** Further adjustment of the main model for maternal pre-pregnancy BMI and population density. Relative difference in percent in semen quality characteristics, testes volume and reproductive hormones in adult sons in relation to nitrate concentration in maternal drinking water.

Semen quality characteristics	Nitrate mg/L	n <sup>a</sup>	Crude	Adjusted for maternal pre-pregnancy BMI <sup>b,c</sup>	Adjusted for population density <sup>b,d</sup>
Volume (mL) <sup>e</sup>		>790			
	≤2		ref	ref	ref
	2-≤5		-3%	0% (-7;7)	0% (-7;7)
	>5		7%	8% (-4;22)	7% (-5;20)
	Per 1 mg/L nitrate		0%	0% (-1;1)	0% (-1;1)
Concentration (million/mL) <sup>f</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-10%	-6% (-16;6)	-6% (-16;6)
	>5		-4%	0% (-16;20)	-1% (-17;19)
	Per 1 mg/L nitrate		-1%	0% (-2;1)	0% (-2;1)
Total sperm count (million) <sup>e</sup>		>790			
	≤2		ref	ref	ref
	2-≤5		-6%	2% (-10;16)	2% (-10;16)
	>5		16%	16% (-3;40)	15% (-4;39)
	Per 1 mg/L nitrate		0%	0% (-1;2)	0% (-1;2)
Normal morphology (%) <sup>g</sup>		>930			
	≤2		ref	ref	ref
	2-≤5		-3%	-2% (-11;8)	-2% (-12;8)
	>5		19%	21% (4;42)	24% (6;46)
	Per 1 mg/L nitrate		1%	1% (-1;2)	1% (-1;2)
Motility, non-progressive and immotile (%) <sup>g,h</sup>		>935			
	≤2		ref	ref	ref
	2-≤5		1%	0% (-5;7)	1% (-5;6)
	>5		-8%	-9% (-17;-1)	-10% (-17;-1)
	Per 1 mg/L nitrate		-1%	-1% (-2;-0)	-1% (-2;-0)
<b>Testes volume</b>					
Average volume (mL) <sup>i</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		1%	1% (-3;6)	2% (-3;7)
	>5		1%	1% (-6;9)	0% (-7;8)
	Per 1 mg/L nitrate		0%	0% (-1;1)	0% (-1;1)
<b>Reproductive hormones</b>					
Estradiol (pmol/L) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-5%	-4% (-11;3)	-4% (-11;3)
	>5		9%	8% (-4;21)	9% (-3;23)
	Per 1 mg/L nitrate		1%	1% (0;2)	1% (0;2)

Follicle stimulating hormone (IU/L) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-1%	-1% (-11;10)	-1% (-12;11)
	>5		-15%	-15% (-26;-1)	-14% (-26;-2)
	Per 1 mg/L nitrate		-1%	-1% (-2%;1)	0% (-2%;1)
Luteninizing hormone (IU/L) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-3%	-2% (-8;5)	-2% (-8;5)
	>5		-8%	-8% (-18;2)	-7% (-17;3)
	Per 1 mg/L nitrate		0%	0% (-1;0)	0% (-1;1)
Sex hormone binding globulin (nmol/L) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-2%	-1% (-6;4)	-1% (-6;4)
	>5		-9%	-8% (-15;0)	-8% (-16;0)
	Per 1 mg/L nitrate		0%	0% (-1;0)	0% (-1;0)
Testosterone (nmol/L) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		-3%	-2% (-6;3)	-2% (-6;3)
	>5		-3%	-3% (-9;3)	-2% (-8;4)
	Per 1 mg/L nitrate		0%	0% (0;1)	0% (0;1)
Free androgen index (%) <sup>j</sup>		>950			
	≤2		ref	ref	ref
	2-≤5		3%	2% (-4;8)	2% (-4;9)
	>5		6%	2% (-5;11)	4% (-3;13)
	Per 1 mg/L nitrate		0%	0% (0;1)	0% (0;1)

a: rounded down to the nearest fifth due to local regulations. b: adjusted for maternal age at delivery, maternal smoking during 1st trimester and highest educational level of parents. c: further adjusted for maternal pre-pregnancy body mass index. d: further adjusted for population density (numbers of neighbors residing within 250 meters of the maternal residential address). e: excluding samples with spillage and further adjusted for abstinence time and place at semen sample collection; at home/at the clinic. f: further adjusted for spillage, abstinence time and place at semen sample collection; at home/at the clinic. g: excluding samples from participants with azoospermia. Further adjusted for spillage, abstinence time and place at semen sample collection; at home/at the clinic. h: further adjusted for interval from ejaculation to analyses of semen sample. i: further adjusted for abstinence time. j: further adjusted for time at the day for collection of blood sample