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Association of In Utero Exposure to Maternal Smoking with Reduced Semen Quality and Testis Size in Adulthood: A Cross-Sectional Study of 1,770 Young Men from the General Population in Five European Countries

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Between 1996 and 1999, the authors invited all young men from five European countries who were undergoing compulsory medical examination for possible military service to participate in a study on male reproductive health. The participation rate was 19% in two cities in Denmark ($n = 889$), 17% in Oslo, Norway ($n = 221$), 13% in Turku, Finland ($n = 313$), 14% in Kaunas, Lithuania ($n = 157$), and 19% in Tartu, Estonia ($n = 190$). Each man provided a semen sample, was examined by a physician, and, in collaboration with his mother, completed a questionnaire about general and reproductive health, current smoking habits, and exposure to smoking in utero. After adjustment for confounding factors, men exposed to smoking in utero had a reduction in sperm concentration of 20.1% (95% confidence interval (CI): 6.8, 33.5) and a reduction in total sperm count of 24.5% (95% CI: 9.5, 39.5) in comparison with unexposed men. Percentages of motile and morphologically normal sperm cells were 1.85 (95% CI: 0.46, 3.23) and 0.64 (95% CI: –0.02, 1.30) percentage points lower, respectively, among men exposed in utero, and exposed men had a 1.15-ml (95% CI: 0.66, 1.64) smaller testis size. The associations were present when data from the study centers were analyzed separately (though not in Lithuania, where only 1% of mothers smoked during pregnancy), although the strength of the association varied. Maternal smoking may have long-term implications for the reproductive health of the offspring. This is another good reason to advise pregnant women to avoid smoking.

pregnancy; prenatal exposure delayed effects; semen; smoking; spermatozoa; sperm count

There has recently been growing interest in and concern about the possible effect of environmental agents on male reproductive health. A number of studies have reported reduced semen quality among men occupationally exposed to various chemicals (1–5), welding (6–8), heat (9–11), or prolonged automobile driving (12). Semen quality has also been studied in relation to various lifestyle factors, such as caffeine and alcohol intake, but no consistent findings have been reported (13–20). Most of these studies have been studies of infertile men. Many early reports found more abnormal sperm cells among smokers, but later studies failed to corroborate this finding (13, 14, 16–19, 21–25). However, a meta-analysis of 20 studies found that smokers had a significant reduction in sperm concentration (26). The investigators concluded that the lack of statistically significant findings in many of the studies may have been due to small sample sizes (26). To our knowledge, only one study has assessed semen quality among men exposed to smoking in utero (27), and no effect on semen characteristics was found. Three studies (28–30) have examined the effect of in utero

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exposure to cigarette smoking on later fecundability, measured as a couple's waiting time to pregnancy, but only two of the studies evaluated male exposure in utero, and the findings were contradictory.

Because of the military draft system in Denmark, Finland, Norway, Lithuania, and Estonia, all young men are required to undergo a medical examination for determination of their fitness for military service. Therefore, these young men are from the general population and have not been selected with respect to their fertility status. Most men undergo the examination at 18–20 years of age, but ages at examination vary. Collaboration with the military health board in each country provided us with a unique opportunity to invite these young men to participate in a study of male reproductive health. Participants answered a questionnaire about reproductive and general health and lifestyle factors, including in utero exposure to cigarette smoking. They provided blood samples for reproductive hormone measurements and semen samples for analysis. The aim of this study was to examine whether exposure to maternal smoking in utero or parental smoking in childhood influenced semen quality in adulthood.

MATERIALS AND METHODS

Populations

All young men from the general populations of five European countries were personally approached by trained staff when they appeared for their compulsory medical examination. The men were approached in Copenhagen and Aalborg, Denmark, between June 1996 and March 1998; in Oslo, Norway, between January 1998 and April 1998; in Kaunas, Lithuania, between October 1997 and June 1998; and in Tartu, Estonia, between October 1997 and June 1999. Men who suffered from chronic diseases (less than 10 percent of the population) were not summoned to the military board examination and therefore were not approached for this study. In Turku, all men were approached by the military board; we received a complete address list for all men who participated between March 1998 and December 1999 and mailed them an invitation to participate in the study. The men ranged in age from 16 years to 27 years.

The trained staff informed the men about the study and handed out written information. The men could fill in the informed consent form for participation and book an appointment immediately or return the consent form by mail. For a young man to participate in the study, he and his mother had to have been born and raised in the country where he was recruited. Participants received economic compensation for their travel expenses and/or lost working hours, according to local traditions. The participation rates at the study centers were 19 percent in Denmark ($n = 889$), 17 percent in Norway ($n = 221$), 13 percent in Finland ($n = 313$), 14 percent in Lithuania ($n = 157$), and 19 percent in Estonia ($n = 190$).

Ethical approval was obtained by local ethical committees in the participating countries. The procedures used were in accordance with the Helsinki Declaration of 1975, as revised in 1983. A detailed description of the semen analysis study has been given elsewhere (31).

Semen analysis

Each man provided a semen sample by masturbating into a wide-mouthed plastic container in a room close to the semen laboratory. The period of abstinence prior to sampling was recorded, and the semen sample was analyzed according to the World Health Organization's 1992 guidelines (32). A smear was taken from all semen samples and stained and preserved. A single physician in Finland assessed morphology using strict criteria (33). The investigator, who was blinded, evaluated 86 percent of the morphology smears. The remaining smears either were damaged in transport or had been fixated insufficiently.

An external quality control program was conducted throughout the study, as described previously (31). A previous study found interobserver variability in semen analysis between different laboratories (34), especially in motility assessment. However, the percentage of progressively moving sperm cells can be used as an outcome measure. The following semen variables were used as outcome variables: semen volume (ml), sperm concentration (millions/ml), percentage of motile sperm cells, percentage of sperm cells with normal morphology, and calculated total sperm count (concentration × volume, in millions).

Physical examination

All participants were examined by a physician. Testicular volumes were determined using a Prader orchidometer, and the mean of both testes was calculated. The possible presence of a varicocele, a hydrocele, or any genital malformation, the location of the testis in the scrotum, and the consistency of the testis and epididymis were recorded. Interobserver differences in testis size between the group members have previously been estimated (in a seminar where all physicians examined the same men) to be 16 percent (35). In addition, weight and height were measured, and body mass index was calculated as weight in kilograms divided by squared height in meters.

Questionnaire

All participants completed a questionnaire that was either submitted to their home address or handed out to them on the day of the military examination. It was returned to the physician at the time of physical examination. The questionnaire included information on previous and/or current diseases and genital conditions, such as cryptorchidism, inguinal hernia, varicocele, epididymitis, gonorrhea, chlamydia, and surgery for torsion of the testis. The men were asked whether they were still in school and, if not, what their highest level of education was and what their current work situation was. They reported on their smoking and alcohol intake during the week before completion of the questionnaire. Smoking habits were reported as the average number of cigarettes, cigars, or pipes smoked per day. Total weekly alcohol intake (number of drinks) was calculated by summarizing beer, wine, and liquor intake. In addition, a section of the questionnaire concerned possible in utero exposures; the men were asked to fill in that section in collaboration with their

mothers, if possible (we recorded whether or not the information was obtained from the mother). The men were asked about their birth weight and length and whether they had been born at term. In addition, they were asked whether their mother had smoked while she was pregnant with them and if their parents had smoked at home (answer categories: neither parent smoked, one smoked, or both smoked).

Statistics

Outcome variables included testis size, semen volume, sperm concentration, total sperm count, and percentage of spermatozoa with normal morphology or motility. Data on sperm concentration and total sperm count were not normally distributed, so the median and interquartile range (25th–75th percentiles) were calculated for these variables. Data on percentages of morphologically normal and motile cells, semen volume, and testis size were all normally distributed, so the mean and standard deviation were calculated for these variables. Diseases of the reproductive organs found to affect any of the outcome variables separately were combined into one variable for each outcome (present or not present).

We compared semen quality and testis size with information obtained from the questionnaire and the physical examination. We then characterized differences in the outcome variables with regard to exposure to smoking in utero and in childhood (explanatory variables) to determine possible confounding factors. Finally, we performed a multiple linear regression analysis, taking into account confounders affecting outcome variables and differently distributed among men exposed to smoking in utero and in childhood. In utero and childhood exposure to smoking were entered into the model as dummy variables. Normally distributed outcome variables were entered directly as continuous variables in the linear multiple regression analysis, whereas sperm concentration and total sperm count were transformed by the use of the natural logarithm to obtain normality. Confounders were excluded stepwise if they were not statistically significant at the 10 percent level. The results are presented with 95 percent confidence intervals. We evaluated the fit of the regression models by testing the residuals for normality and by inspecting the residual plots.

Since birth weight might be an intermediary factor in the pathway between in utero exposure to smoke and reduced semen quality and testis size, we calculated the contribution of birth weight to reduced semen quality and testis size by dividing the standardized beta coefficients for in utero smoking exposure with and without birth weight (as a continuous variable) in the model and subtracting this from 1.

No significant interaction between smoking exposure in utero and smoking exposure in childhood was found (a dummy variable for different exposure points was added to regression analyses and was not significant). Accordingly, no interaction terms were entered into the multiple logistic regression analyses, but smoking exposure in utero was entered as a confounder in the model when childhood exposure was the explanatory variable and vice versa.

RESULTS

The mean testis size among the men was 20.7 ml (standard deviation, 5.0), and the mean semen volume was 3.1 ml (standard deviation, 1.5). The median sperm concentration and total sperm count were 52 million/ml (25th–75th percentiles, 25–91) and 149 million (25th–75th percentiles, 60–274), respectively, whereas mean percentages of motile and morphologically normal spermatozoa were 66.0 (standard deviation, 12.4) and 7.6 (standard deviation, 5.3), respectively (table 1). The questionnaire and the physical examination were reasonably complete (the percentage of missing data was less than 10 for all relevant variables, including smoking exposure in utero and in childhood), apart from the questions on birth weight, for which data were missing on approximately 25–50 percent of the questionnaires. We decided not to include the information on birth weight in the initial regression analysis, since it would have reduced the power of the study considerably.

Sperm concentration and total sperm count were reduced if a varicocele or hydrocele was found at the physical examination, if the testes were abnormally hard or soft, or if the man reported having had cryptorchidism or undergoing surgery for torsion of the testis. Testis size was reduced if a varicocele was found at the physical examination, if the testes were high in the scrotum, if the testes were hard or soft, or if the man reported having been treated for cryptorchidism. The percentages of morphologically normal cells and motile sperm cells were reduced if the man reported having had cryptorchidism or if the testes were hard or soft. The percentages of motile sperm cells were also reduced if the man had undergone surgery for torsion of the testes, and the percentage of morphologically normal cells was reduced when the man reported having had gonorrhea or chlamydia. Semen volume was not significantly affected by any of these conditions. Data on any diseases found to affect semen quality or testis size were combined to create one variable for each outcome (present or not present).

Table 1 summarizes information from the men exposed to smoking in utero or in childhood. Current smoking and smoking exposure in utero and in childhood were correlated. Linear regression was performed with semen quality and testis size as dependent variables and exposure in utero and in childhood as explanatory variables. Before adjustment, men exposed to smoking in utero and in childhood had a smaller testis size, a lower sperm concentration and total sperm count, and fewer motile and morphologically normal sperm cells than unexposed men (table 2). After the data were controlled for body mass index, study center, diseases of the reproductive organs related to sperm concentration or total sperm count, period of abstinence (transformed by the use of the natural logarithm), and, for total sperm count, age and season, men exposed to smoking in utero had reductions in sperm concentration and total sperm count of 20.1 percent (95 percent confidence interval (CI): 6.8, 33.5) and 24.5 percent (95 percent CI: 9.5, 39.5), respectively, compared with unexposed men. Men exposed to smoking in utero had a 1.15 ml (95 percent CI: 0.66, 1.64) smaller testis size after data were controlled for body mass index, study center, age, still being in school, and diseases related to size. Percentages

TABLE 1. Data on demographic and male reproductive factors (semen quality and testis size) according to passive exposure to smoking in utero or in childhood among potential military conscripts from five European countries, 1996–1999

Variable	Total (n = 1,770)		Passive smoking exposure			
			In utero exposure to smoking		Smoking by one or both parents in childhood	
	No.	%	No	Yes	No	Yes
Male reproductive factors						
Mean testis size* (ml)	20.7 (5.0)†	98.7	21.3 (5.0)	19.1 (4.4)	21.6 (4.8)	20.2 (4.9)
Mean semen volume (ml)	3.1 (1.5)	100.0	3.1 (1.5)	3.0 (1.5)	3.2 (1.4)	3.0 (1.5)
Median sperm concentration (millions/ml)	52 (25–91)‡	100.0	56 (30–95)	41 (19–78)	59 (33–98)	47 (23–84)
Median total sperm count (millions)	149 (60–274)	100.0	163 (70–287)	113 (45–218)	180 (87–294)	127 (53–250)
Mean % of motile sperm	66.0 (12.4)	99.2	67.1 (11.9)	63.0 (13.2)	66.5 (11.7)	65.7 (12.8)
Mean % of morphologically normal cells	7.6 (5.3)	87.3	7.8 (5.4)	7.1 (5.1)	8.4 (5.4)	7.1 (5.2)
Period of abstinence (hours)						
0–95	1,326	76.2	72.6	27.4	39.0	61.0
≥96	415	23.8	78.1	21.9	36.9	63.1
Season						
April–September	376	21.4	81.6	18.4	47.2	52.8
October–March	1,380	78.6	71.4	28.6	35.8	64.2
Questionnaire information						
Study center						
Copenhagen or Aalborg, Denmark	889	50.2	59.2	40.8	24.1	75.9
Tartu, Estonia	190	10.7	94.2	5.8	45.9	54.1
Kaunas, Lithuania	157	8.9	98.7	1.3	41.8	58.2
Oslo, Norway	221	12.5	75.1	24.9	38.8	61.2
Turku, Finland	313	17.7	88.5	11.5	71.3	28.7
Age (years)						
<20	1,443	82.5	73.6	26.4	39.7	60.3
≥20	307	17.5	74.3	25.7	31.5	68.5

Table continues

of motile and morphologically normal sperm cells were 1.85 (95 percent CI: 0.46, 3.23) and 0.64 (95 percent CI: –0.02, 1.30) percentage points lower, respectively, among men exposed in utero than in unexposed men after control for study center, age, diseases of the reproductive organs related to motility or morphology, and, for motility, smoking. The finding of 0.64 percentage points' fewer morphologically normal cells should be interpreted in relation to the mean portion of morphologically normal sperm cells in the entire sample, which was only 7.6 percent; thus, this represents a considerable reduction. Exposure to smoking in childhood had an insignificant effect on semen quality and testis size after adjustment for potential confounders, including in utero exposure to smoking (table 2).

We repeated the analyses including only those men who reported that the information on smoking exposure had been obtained from their mothers (n = 917). The same magnitude of effect was found: Sperm concentration and total sperm count were reduced 16 percent (95 percent CI: –1, 33) and 18 percent (95 percent CI: –1, 37), respectively. Percentages of motile and morphologically normal sperm cells were 1.3 (95 percent CI: –0.6, 3.2) and 1.0 (95 percent CI: 0.1, 1.9) percentage points lower, respectively, among men exposed in utero, and exposed men had a 0.9-ml (95 percent CI: 0.2, 1.5) smaller testis size. The percentages of men with information obtained from their mothers varied greatly between study centers, from 0 in Lithuania to 83 in Finland (table 3). In addition, we conducted the analyses for the five countries separately to test whether the association was found in both populations with many exposed men and populations with few exposed men (table 3). The reduced semen quality and testis size associated with in utero exposure to smoking were found in all of the countries except Lithuania, where fewer than 2 percent of the mothers had smoked during pregnancy. However, the magnitudes of the associations varied between centers. We repeated the analyses after excluding mothers from Lithuania; this did not change the results.

We repeated the analyses without adjustment for diseases of the reproductive organs, since in utero exposure to smoking may cause these conditions and they may thus be intermediary factors. The associations were even stronger without the adjustment. In addition, we performed multiple regression analyses in the subset of data for which information on birth weight was available. Birth weight only significantly reduced semen quality and testis size when in utero exposure to smoking was excluded from the model and vice versa. This indicates that birth weight might be an intermediate factor in the relation between in utero exposure to smoking and semen quality. Birth weight accounted, respectively, for 21, 18, 25, 13, and 6 percent of the reductions in

TABLE 1. Continued

Variable	Total (n = 1,770)		Passive smoking exposure			
			In utero exposure to smoking		Smoking by one or both parents in childhood	
	No.	%	No	Yes	No	Yes
Body mass index§						
<20	265	15.1	70.9	29.1	35.5	64.5
20–25	1,205	68.7	75.4	24.6	40.5	59.5
≥26	283	16.1	68.9	31.1	30.2	69.8
Still in school						
No	600	34.1	66.2	33.8	31.5	68.5
Yes	1,158	65.9	77.3	22.7	41.7	58.3
Alcohol intake (drinks/week)						
0–19	1,254	78.8	74.5	25.5	40.1	59.9
≥20	337	21.2	67.4	32.6	29.6	70.4
Smoking						
No	734	41.6	72.3	27.7	34.5	65.5
Yes	1,029	58.4	74.4	25.6	40.7	59.3
In utero/childhood exposures						
Smoking exposure in utero						
No	1,303	73.6			50.2	49.8
Yes	467	26.4			5.0	95.0
Smoking exposure in childhood						
Neither parent smoked	667	38.2	96.6	3.4		
One parent smoked	628	35.9	79.0	21.0		
Both parents smoked	453	25.9	31.8	68.2		
Birth weight (g)						
<3,000	154	14.5	57.1	42.9	33.3	66.7
≥3,000	906	85.5	73.7	26.3	40.9	59.1

* Calculated as the mean of right and left testis size.
† Single numbers in parentheses, standard deviation.
‡ Ranges in parentheses, 25th–75th percentiles.
§ Weight (kg)/height $(m)^2$.

testis size, sperm concentration, total sperm count, percentage of motile sperm cells, and percentage of morphologically normal sperm cells.

We evaluated the validity of the smoking exposure variable by calculating mean birth weights among men exposed to smoking in utero and men not exposed. In the 60 percent of men for whom information on birth weight was available, the mean birth weight among the exposed was 3,361 g—267 g less than that among the unexposed (3,628 g).

DISCUSSION

In this study of more than 1,700 men from the general population of five European countries, we found reduced sperm concentrations and sperm counts, a smaller testis size, and fewer motile and morphologically normal sperm cells among men exposed to cigarette smoking in utero than among men not exposed. The results were adjusted for differences in other factors related to semen quality, including the man's present smoking habits and exposure to passive smoking in childhood, which did not reduce semen quality after adjustment for in utero exposure to smoking. Our findings seem to be biologically coherent, since it is classical andrologic knowledge that smaller testis size is associated with decreased sperm production (36). Furthermore, the associations between smoking and reduced reproductive parameters were present when data from the participating study centers were analyzed separately. Therefore, we believe that our finding of an association between in utero exposure to smoking and deteriorated reproductive health is real.

We found no independent effect of exposure to smoking in childhood, but this information was only classified as whether one or two parents had smoked at home. Therefore, it was impossible for us to determine the exact magnitude and timing of this exposure.

An effect of birth weight was found only when in utero exposure to smoking was excluded from the model and vice versa. This may indicate that birth weight is an intermediate factor in the relation between in utero exposure to smoking and semen quality and testis size. Nevertheless, birth weight accounted for less than 25 percent of the reduction in semen quality and testis size. The factors causing low birth weight may also be involved in impairment of the gonads during

TABLE 2. Unadjusted and adjusted results from linear regression analyses of semen quality and testis size among European men exposed to smoking in utero or in childhood as compared with unexposed men, 1996–1999*

	Change in testes size (ml)		Change in semen volume (ml)		Change in sperm concentration (%)		Change in total sperm count (%)		Change in percentage of motile sperm cells (percentage points)		Change in percentage of morphologically normal cells (percentage points)	
	β	95% CI†	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Unadjusted results												
In utero exposure to maternal smoking	–2.23	–2.75, –1.71	–0.11	–0.27, 0.04	–36.6	–49.0, –24.3	–42.1	–56.0, –28.2	–4.02	–5.33, –2.72	–0.75	–1.36, –0.15
Smoking by one parent in childhood	–1.14	–1.67, –0.60	–0.14	–0.29, 0.02	–21.1	–33.8, –8.4	–28.9	–43.1, –14.7	0.33	–1.02, 1.68	–1.36	–1.98, –0.74
Smoking by both parents in childhood	–1.79	–2.38, –1.21	–0.25	–0.43, –0.08	–34.9	–48.8, –21.0	–47.2	–62.8, –31.6	–2.42	–3.90, –0.94	–1.02	–1.70, –0.34
Adjusted results												
In utero exposure to maternal smoking	–1.15‡	–1.64, –0.66	–0.03§	–0.20, 0.13	–20.1¶	–33.5, –6.8	–24.5#	–39.5, –9.5	–1.85**	–3.23, –0.46	–0.64††	–1.30, 0.02
Smoking by one parent in childhood	–0.69‡‡	–1.20, –0.18	–0.08‡‡	–0.25, 0.09	–10.7‡‡	–24.3, 2.9	–15.0‡‡	–30.3, 0.2	0.24‡‡	–1.18, 1.66	–0.74‡‡	–1.42, –0.06
Smoking by both parents in childhood	0.06‡‡	–0.72, 0.59	–0.23‡‡	–0.45, –0.01	–8.7‡‡	–26.5, 9.1	–19.3‡‡	–39.3, 0.6	0.16‡‡	–1.69, 2.00	–0.38‡‡	–1.28, 0.51

* A change in sperm concentration of –20.1% and a change in motility of –1.85 mean that exposed men had a 20.1% lower sperm concentration and 1.85 percentage points' fewer motile sperm cells, respectively, than unexposed men.

† CI, confidence interval.

‡ Adjusted for body mass index, study center, age, still being in school, and diseases of the reproductive organs related to testis size.

§ Adjusted for body mass index, study center, age, and period of abstinence (transformed by the use of the natural logarithm).

¶ Adjusted for body mass index, study center, diseases of the reproductive organs related to sperm concentration, and period of abstinence (transformed by the use of the natural logarithm).

Adjusted for body mass index, study center, age, diseases of the reproductive organs related to total sperm count, season at delivery, and period of abstinence (transformed by the use of the natural logarithm).

** Adjusted for study center, age, current smoking, and diseases of the reproductive organs related to percentage of motile sperm cells.

†† Adjusted for study center, age, and diseases of the reproductive organs related to percentage of morphologically normal sperm cells. Men exposed in utero had 0.64 percentage points' fewer morphologically normal sperm cells, but the mean percentage of normal cells was only 7.6.

‡‡ Adjusted for the same variables as in the model above plus exposure to smoking in utero.

TABLE 3. Participation rates, percentages of men exposed to smoking in utero and in childhood, and adjusted changes in semen quality and testis size among men exposed to smoking in utero in the five European study locations, 1996–1999

	Study center				
	Copenhagen or Aalborg, Denmark	Tartu, Estonia	Kaunas, Lithuania	Oslo, Norway	Turku, Finland
No. of participants	889	190	157	221	313
Participation rate (%)	19	19	14	17	13
Exposure to smoking in utero (%)					
No	59	94	99	75	88
Yes	41	6	1	25	12
Information obtained from mother (%)					
No	7	38	0	0	14
Yes	53	32	0	54	83
Missing data	40	30	100	46	3
Smoking exposure in childhood (%)					
Neither parent smoked	24	44	41	39	71
One parent smoked	38	41	50	36	17
Both parents smoked	37	11	6	25	12
Missing data	1	4	3	1	1
Birth weight (%)					
>3,000 g	11	4	2	6	11
≥3,000 g	49	32	22	67	71
Missing data	40	64	76	26	18
Adjusted change in semen parameters among men exposed to smoking in utero					
Testis size (ml)*	–1.4 (–2.0, –0.8)†	– 0.1 (–3.3, 1.2)	–0.8 (–8.1, 6.5)	–0.2 (–1.6, 1.3)	–0.5 (–2.0, 0.9)
Semen volume (ml)‡	–0.06 (–0.3, 0.1)	–0.5 (–1.5, 0.4)	–1.6 (–3.5, 0.8)	0.1 (–0.3, 0.5)	0.1 (–0.4, 0.6)
Sperm concentration (%)§	–22 (–41, –5)	–14 (–67, 40)	110 (–5.1, 225)	–17 (–50, 16)	–16 (–55, 23)
Total sperm count (%)¶	–28 (–48, –8)	–17 (–90, 57)	–2 (–167, 163)	–20 (–56, 16)	–13 (–55, 29)
Percentage of motile sperm cells (percentage points)#	–2.0 (–3.7, –0.4)	–4.9 (–12.6, 2.9)	3.6 (–12.0, 19.6)	–1.8 (–4.7, 1.2)	–0.6 (–5.4, 4.2)
Percentage of morphologically normal sperm cells (percentage points)**	–0.5 (–1.3, 0.3)	–3.9 (–8.1, 0.3)	–1.3 (–7.2, 4.5)	0.3 (–1.4, 2.0)	–1.6 (–3.7, 0.5)

* Adjusted for body mass index, study center, age, still being in school, and diseases of the reproductive organs related to testis size.
† Numbers in parentheses, 95% confidence interval.
‡ Adjusted for body mass index, study center, age, and period of abstinence (transformed by the use of the natural logarithm).
§ Adjusted for body mass index, study center, diseases of the reproductive organs related to sperm concentration, and period of abstinence (transformed by the use of the natural logarithm).
¶ Adjusted for body mass index, study center, age, diseases of the reproductive organs related to total sperm count, season at delivery, and period of abstinence (transformed by the use of the natural logarithm).
# Adjusted for study center, age, current smoking, and diseases of the reproductive organs related to percentage of motile sperm cells.
** Adjusted for study center, age, and diseases of the reproductive organs related to percentage of morphologically normal sperm cells.

development. Previous studies on fertility in children with low birth weight have been conflicting (37, 38). None of the previous investigators controlled for the effect of in utero exposure to smoking. Since children with low birth weight may be more likely to have cryptorchidism and a low body mass index (39), conditions that may cause reduced semen quality, we repeated the analyses after exclusion of men with these conditions. An effect of in utero exposure to smoking on semen quality was still present in this group, a finding that seems to be in line with the assumption that birth weight is an intermediary factor.

Studies have found that both male and female mouse fetuses exposed to polycyclic aromatic hydrocarbons, the major toxic compounds found in cigarette smoke, have reduced fertility in adulthood (40). Polycyclic aromatic hydrocarbons increase apoptosis in oocytes when administered in utero and in adulthood and may also operate in testicular germ cells (41, 42). One study found an increased time to pregnancy among men prenatally exposed to cigarette smoking, but another could not confirm this finding (28–30). These reports raised the question of whether exposure of the human male fetus to tobacco components may reduce adult reproductive capacity irreversibly. To our knowledge, only one study has examined semen quality among men exposed to smoking in utero (27), and no effect on semen characteristics was found. However, in that study, the mothers of the men participated in a randomized clinical trial of diethylstilbestrol, which may itself reduce semen quality (43, 44). Numerous studies included in a meta-

analysis (26) examined the effect of current smoking and found an effect on semen quality. This could not be confirmed in our study. In fact, prenatal smoking exposure was the strongest predictor of poor semen quality, and current smoking had no independent effect, even though smoking exposures in utero, in childhood, and in adulthood were strongly correlated. In addition, the effect of in utero exposure on semen quality and testis size was also found among nonsmoking men. Since previous studies have not taken in utero exposure to smoking into account, the reported relation between semen quality and current smoking may be caused by in utero exposure.

There were limitations in our study. Firstly, the participation rate was low (13–19 percent), and this may have caused a selection bias of unknown direction and magnitude. However, the men had essentially no prior knowledge of their own fertility potential, and therefore this is unlikely to have affected their motivation to participate. Bias in relation to the reported associations between in utero smoking exposure and semen quality is only likely if in-utero-exposed men with poor semen quality were oversampled. This is unlikely, since in utero exposure to smoking is not an established risk factor for poor semen quality.

Secondly, the quality of the information on prenatal smoking exposure in this study may be questioned, since we relied on retrospectively collected data. When we asked the men whether they had obtained the information on in utero exposure directly from their mothers and included only those men who indicated this, we found the same results. In addition, the mean birth weight among men exposed in utero was 264 g less than that among the unexposed; this corresponds to the deficit in birth weight from smoking found in other studies (45), thus indicating that the validity of the data on smoking exposure was good. However, this only indicates that the smoking information was valid for the 60 percent of the men who had information on birth weight. Some mothers may have a selective memory about their smoking habits, but since they answered the questionnaire before knowing the outcome of their sons' semen analysis, this is unlikely to have affected the results. Ninety percent of the men with birth weight information had information on smoking habits from their mothers, and this information was probably more valid than the rest. In the two Baltic countries, the proportion of mothers who smoked was very low (<6 percent), and thus smoking in these countries may have been underreported. Nevertheless, underreporting would have misclassified exposed men as unexposed and thus caused underestimation of the true association.

The association between prenatal smoking exposure and semen quality could be due to confounding by other prenatal factors related to smoking, such as alcohol intake, diet, or drug use. We did not collect information on these factors. Present exposures or parental exposures (including an effect of paternal smoking mediated through fathers' sperm) could also have confounded the association. We collected information about a wide range of present exposures with a possible effect on semen quality, such as smoking, alcohol intake, and education, but none of these factors affected semen quality, and they were controlled for in the multiple regression analysis. We collected information about intake during the week before questioning, because this has been reported to be more reliable than average exposure, but it does not measure exposure during the entire period of semen production. In addition, variation in smoking habits from week to week is low. Semen quality may vary with season, and it is affected by period of abstinence (46). However, the majority (78 percent) of the semen samples in this study were provided during a short period from October to February, and since we controlled for differences in period of abstinence, these factors are unlikely to explain our findings. No seasonal variation was observed at one of the centers (Turku), where samples were equally distributed over different seasons (31).

Thirdly, it is well known that interobserver variability in semen analysis between different laboratories may be present (34), especially in motility assessment, and this may have influenced our findings. Variation in physical examination (in testis size) results between physicians from these centers has previously been reported (35). However, we adjusted for differences between centers in the multiple regression analyses, and the relation between in utero exposure to smoking and semen quality and testis size was found independently in each country (except for Lithuania, where less than 2 percent of the pregnant women smoked). In addition, the technicians were blinded to the exposure information, and the same physician assessed all morphology slides. An external quality control program conducted throughout the study monitored semen analysis techniques. Thus, between-laboratory and physician variation are unlikely explanations for our findings.

In conclusion, we found reduced semen quality and a smaller testis size among young men exposed to smoking in utero in comparison with unexposed men, but we detected no independent effect of exposure to passive smoking in childhood. We do not believe that selection bias can explain our findings. To our knowledge, this study is the first to have shown a correlation between semen quality, testis size, and in utero exposure to cigarette smoking. Clearly, these findings must be confirmed by other studies. However, it seems reasonable to advise pregnant women to avoid smoking, since it not only may cause adverse birth outcomes but also may have long-term implications for the reproductive health of the offspring.

Note added in proof: *Since the submission of this paper, Storgaard et al. (47) have published results showing a 48 percent reduction in semen concentration among sons exposed to maternal smoking of more than 10 cigarettes per day.*

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