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Original Contribution

Is Prenatal Exposure to Tobacco Smoking a Cause of Poor Semen Quality? A Follow-up Study

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A few studies indicate that exposure to maternal smoking during fetal life decreases semen quality in adult life, but the results are inconsistent and retrospectively collected smoking data were used in most studies. From a Danish pregnancy cohort established in 1984–1987, 347 of 5,109 sons were selected according to their exposure to tobacco smoke in fetal life. From February 2005 to January 2006, a semen sample from the 347 men was analyzed for conventional semen characteristics according to standardized criteria by using a mobile laboratory. The authors found an inverse association between maternal smoking during pregnancy and total sperm count ($p = 0.002$). Men exposed to more than 19 cigarettes daily during pregnancy had approximately 19% lower semen volume ($p = 0.04$), 38% lower total sperm count ($p = 0.11$), and 17% lower sperm concentration ($p = 0.47$) compared with unexposed men. The odds ratio for oligospermia was 2.16 (95% confidence interval: 0.68, 6.87) among exposed men compared with the unexposed. No associations were found for sperm motility or morphology. These results indicate that prenatal exposure to tobacco smoke may have an adverse effect on semen quality and, if these associations are causal, they could explain some of the reported differences between populations and secular changes in semen quality.

oligospermia; prenatal exposure delayed effects; semen; smoking; spermatozoa; sperm count; sperm motility

Abbreviations: CI, confidence interval; HH42, Healthy Habits for Two.

A possible decrease in semen quality over time has been much debated, but geographic differences in semen quality are evident (1). Exposure to environmental hormonal disruptors has been suggested as a possible explanation for these differences, but prenatal exposure to tobacco smoke may be a better causal candidate because smoking habits correlate better with the existing epidemiologic data (2).

A possible effect of prenatal tobacco smoking on semen quality in adult life has been investigated in only a few studies. The first study examined sons of mothers who participated in a randomized clinical trial on diethylstilbestrol use during pregnancy. No effect of prenatal exposure to tobacco was found (3). Three succeeding studies based on a large exposure contrast reported a detrimental effect of maternal smoking during pregnancy (2, 4, 5). Storgaard et al. (2) found a 51 percent lower total sperm count among sons of mothers who had smoked more than 10 cigarettes per day during pregnancy compared with sons of nonsmoking mothers. Jensen et al. (4) found that men exposed to maternal smoking in fetal life had a 25 percent lower total sperm count than did unexposed men. Finally, Jensen et al. (5) reported tendencies toward a lower sperm concentration and total sperm count with increased prenatal tobacco exposure. Men exposed to 10 or more cigarettes per day in

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fetal life had an odds ratio of 2.6 for a sperm concentration below 20 million/ml.

All the previous studies used retrospectively collected smoking data from either the sons or the mothers. We conducted a population-based follow-up study in a population with many heavy smokers, and we use prospective data on smoking. The aim of the present study is to investigate the possible association between prenatal smoking exposure and semen characteristics in young adults.

MATERIALS AND METHODS

Population

The participants were sons of mothers who during pregnancy were included in the Healthy Habits for Two (HH42) cohort. The cohort was established from April 1984 to April 1987 in two Danish municipalities (Aalborg and Odense), and 11,980 pregnant women who gave birth to singleton infants (more than 80 percent of all invited) participated. They provided information on lifestyle factors during pregnancy, including smoking (6).

These mothers' 5,109 sons, who were alive and living in Denmark by December 2004, were identified in the Danish Civil Registration System. Pregnancy reports showed that 62.3 percent had not been prenatally exposed to maternal smoking, 18.7 percent had been exposed to 1–9 cigarettes/day (light smoking), 17.5 percent had been exposed to 10–19 cigarettes/day (medium smoking), and 1.5 percent had mothers who reported smoking 20 or more cigarettes/day (heavy smoking) during pregnancy. The aim was to enroll 100 unexposed men, 100 exposed to light smoking, 100 exposed to medium smoking, and 50 exposed to heavy smoking. Letters of invitation were sent first to the oldest. Men living close to either Aalborg (north of Jutland) or Odense (Funen) had priority, starting at the city centers and up to approximately 30 km from these centers (55 percent of the 5,109 men). Because of a limited number of men heavily exposed prenatally, an expanded geographic recruitment area was used for this group. Finally, men whose mothers had reported information on health-related issues from childhood to adolescence were given priority. This information was obtained from the mothers by means of self-administered questionnaires when their children were 16–19 years of age, and data were available for 74 percent of all participants.

The selected participants were 18–21 years of age and received economic compensation (about US $85) for taking part in the semen-sampling study that started in February 2005 and ended in January 2006. Men with severe handicaps or congenital syndromes, such as spastic paraplegia or Down's syndrome, were excluded, as were men with metabolic diseases or psychiatric disorders. The study was approved by the regional ethics committee (registered number 20040174), and participation was made conditional on written, informed consent.

A total of 347 (49 percent) men participated in the study (figure 1). Of the 100 men that declined participation by mail or telephone (figure 1), 82 provided some information on their health. Additionally, we have information on 134 nonparticipants who filled in a self-completed questionnaire, which was sent to all sons and daughters in the HH42 cohort in 2005.

Assessment of prenatal tobacco exposure

Information on maternal smoking during pregnancy was obtained in the HH42 study by self-administered questionnaire handed out by the midwives around the 36th week of gestation, filled out at home, and returned in sealed envelopes to the university's research department. Questions were asked on smoking habits during the year before pregnancy, smoking in early pregnancy, current smoking, type and brand of tobacco smoked, change in smoking habits during pregnancy, and husbands' smoking habits. In the present study, maternal smoking in late pregnancy was used as the primary exposure variable. The mothers were asked, ''How much do you currently smoke on average?'', and they were given the following six response categories: ''do not smoke now,'' ''smoke 1–4 cigarettes daily,'' ''smoke 5–9 cigarettes daily,'' ''smoke 10–14 cigarettes daily,'' ''smoke 15–19 cigarettes daily,'' or ''smoke 20 or more cigarettes daily.''

Data extracted from obstetric records provided information on birth weight.

Data collected from the sons

The participants were instructed to abstain from having ejaculation for 24 hours before providing the semen sample by masturbating into a plastic container at home. The containers were then to be kept close to the body during transportation to avoid cooling and were brought to the mobile laboratory where a trained medical laboratory technician performed the initial semen analysis. The participants completed questionnaires on reproductive, medical, and lifestyle factors (smoking, alcohol consumption, and coffee consumption), time and date of the preceding ejaculation, spillage during the collection, and fever within the last 3 months. In addition, each participant recorded his weight and height, from which we calculated his body mass index (weight (kg)/height $(m)^2$).

Semen analysis

Semen analyses were performed blinded to prenatal tobacco exposure levels. Semen volume was estimated by its weight (1 g = 1 ml). After liquefaction in a heated chamber at 37°C, sperm motility was assessed by classifying 200 sperm cells within each of two fresh drops of semen as either motile (grades A and B) or immotile (grades C and D) (7). Examination of 82.4 percent of the samples was initiated within the first hour, where it has been shown that the motility is stable (8), and examination of 99.7 percent of the samples was initiated within 2 hours. The sperm concentration of an appropriate dilution was counted in duplicate by use of an improved Neubauer hemocytometer (Paul Marienfeld, Bad Mergentheim, Germany). Smears were air dried, fixed in 95 percent ethanol, and stained with

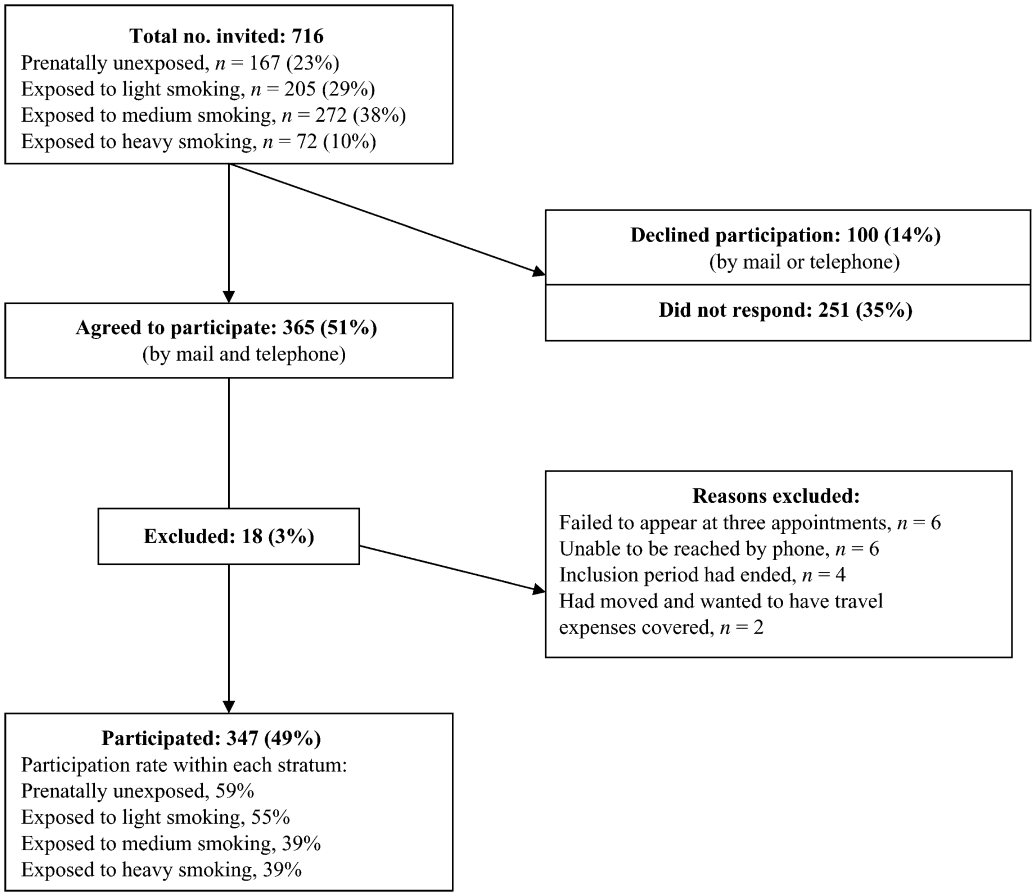

FIGURE 1. Flow chart for study population of young Danish men, 2005–2006.

a modification of Papanicolaou stain. Sperm morphology was determined by strict criteria (9).

The analysis for sperm motility and concentration was performed in accordance with the *WHO Laboratory Manual for the Examination of Human Semen-Cervical Mucus Interaction* (7). The laboratory took part in the European Society for Human Reproduction and Embryology (ESHRE) Nordic external quality control program, and all control tests were within the limits set by this organization.

Testicular volume

The participants were instructed to measure their testicular volumes themselves using a Prader orchidometer at the study site. We calculated the mean value for both testes, and information on testicular volume was available for 201 (58 percent) participants.

Statistical analysis

Crude median and 25th and 75th percentiles were calculated for all semen outcome variables (table 2). For each of the outcome variables, we performed generalized linear regressions, using the three strata of prenatal exposure to tobacco (1–9, 10–19, >19 cigarettes/day) as a categorically coded explanatory variable with unexposed as the referent. When testing for trend, we entered the six original smoking strata as a continuous explanatory variable. Data on semen volume, sperm concentration, total sperm count, percentage of morphologic normal sperm, and testicular volume were cubic-root transformed, and data on percentage of motile sperm were logit transformed to normalize the distribution of the residuals. We evaluated the fit of the regression models by inspecting the residual and leverage plots. The confounder-adjusted results are presented as back-transformed means with 95 percent confidence intervals.

Participants who reported spillage during sampling (n = 88) were excluded from all statistical analysis on sperm volume and on total sperm count.

The following possible confounders were identified and a priori grouped into covariates that were kept in all the models: abstinence time (categorical: <48 hours, 48 hours–5 days, and >5 days) and current smoking (yes/no), and additionally, in the motility model, time from ejaculation to analysis. The remaining covariates in table 1 were evaluated by forward stepwise entering in the model. If they did not change the estimate of interest by at least 10 percent, they were not kept in the final model. Other analytical

TABLE 1. Characteristics of 347 young Danish men stratified by level of maternal smoking during pregnancy, 2005–2006

	Maternal smoking during pregnancy (cigarettes/day)			
	Nonsmoker ($n = 99$)	1–9 ($n = 113$)	10–19 ($n = 107$)	>19 ($n = 28$)
Person-related characteristics of sons				
Body mass index, kg/m^2 (mean (SD*))	22.9 (3.1)	22.8 (3.3)	24 (4.0)	24.5 (4.3)
Birth weight, g (mean (SD))	3,644 (502)	3,532 (548)	3,312 (464)	3,339 (456)
History of diseases in reproductive organs† (no. (%))	9 (9)	17 (15)	24 (22)	9 (32)
Cigarette smoking daily (no. (%))	36 (36)	47 (42)	37 (35)	17 (61)
Alcohol consumption weekly (no. (%))	39 (39)	64 (57)	49 (46)	13 (46)
Coffee consumption daily (no. (%))	17 (17)	17 (15)	21 (20)	4 (14)
Person-related characteristics of mothers				
Maternal age, years (mean (SD))	27.8 (4.0)	27.1 (4.7)	28.2 (4.5)	26.8 (5.3)
Maternal alcohol consumption during pregnancy (no. (%))	81 (82)	97 (86)	90 (84)	20 (71)
Maternal coffee consumption of >3 cups‡/day during pregnancy (no. (%))	18 (18)	32 (28)	63 (59)	22 (79)
Socioeconomic groups I–III§ (combined with husband, highest rating) (no. (%))	50 (51)	59 (52)	45 (42)	5 (18)
Semen-related characteristics				
Season (no. (%))				
April-September	48 (48)	64 (57)	76 (71)	26 (93)
October-March	51 (52)	49 (43)	31 (29)	2 (7)
Duration of abstinence, days, back transformed (mean (SD))	2.6 (0.02)	2.7 (0.02)	2.7 (0.08)	2.6 (0.02)
Minutes from ejaculation to start of analysis, back transformed (mean (SD))	50 (0.08)	50 (0.05)	52 (0.07)	47 (0.03)
Recent fever (no. (%))	14 (14)	20 (18)	15 (14)	7 (25)
Spillage at sampling (no. (%))	23 (23)	28 (25)	30 (28)	7 (25)

* SD, standard deviation.

† History of diseases in the reproductive organs includes cryptorchidism, hypospadias, varicocele, hydrocele, orchiditis, and chlamydial infection combined into one variable (present or not present).

‡ Three cups = 0.7 liter.

§ Socioeconomic groups I–III: white-collar workers; socioeconomic groups IV–VII: blue-collar workers, students, and people unemployed.

models (10) were used as well to examine the robustness of the findings.

Finally, the sperm concentration was dichotomized into oligospermia (>0–<20 million/ml) and normospermia (≥20 million/ml). Logistic regression was used to calculate the crude and adjusted odds ratios for oligospermia in relation to prenatal tobacco exposure, by use of the nonexposed men as referent.

All statistical analyses were performed by STATA, version 8.2, software (StataCorp LP, College Station, Texas). A two-tailed probability level of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of the 347 participants according to maternal smoking during pregnancy are given in table 1. Compared with the nonexposed, men prenatally exposed to tobacco smoking had a lower birth weight, had a higher body mass index as adults, were more often smokers themselves, had more often had diseases of the reproductive organs (cryptorchidism, hypospadias, varicocele, hydrocele, orchiditis, or chlamydial infection), and had mothers who drank coffee during pregnancy. They also more often had their semen collected in the summer period than did the nonexposed. Finally, their parents were less often white-collar workers.

We found an inverse trend between prenatal exposure to tobacco smoke and crude median semen volume, sperm concentration, and total sperm count (table 2). After adjustment for potential confounders, the trend between maternal smoking during pregnancy and semen volume and sperm concentration attenuated ($p_{trend} = 0.11$ and $p_{trend} = 0.13$, respectively), but the significant trend of decreased total

TABLE 2. Semen characteristics and testicular size for 347 young Danish men according to level of maternal smoking during pregnancy, 2005–2006*,†

Parameter	Maternal smoking during pregnancy (cigarettes/day)								p_{trend}‡	
	Nonsmoker ($n = 99$)		1–9 ($n = 113$)		10–19 ($n = 107$)		>19 ($n = 28$)			
	Median (25th–75th percentiles)	Mean (95% CI§), adjusted back transformed	Median (25th–75th percentiles)	Mean (95% CI), adjusted back transformed	Median (25th–75th percentiles)	Mean (95% CI), adjusted back transformed	Median (25th–75th percentiles)	Mean (95% CI), adjusted back transformed	Median (25th–75th percentiles)	Mean (95% CI), adjusted back transformed
Sperm concentration (million/ml)	49 (24–85)	53 (39, 68) (a-g)	50 (18–94)	50 (36, 66) (a-g)	36 (20–74)	43 (29, 62) (a-g)	33 (12–75)	44 (24, 74) (a-g)	0.03	0.13
Semen volume (ml)	3.3 (2.5–4.2)	3.6 (3.2, 4.0) (a-c, h)	2.9 (2.2–3.8)	3.1 (2.7, 3.5) (a-c, h)	2.5 (2.0–4.0)	3.1 (2.7, 3.6) (a-c, h)	2.6 (1.7–3.0)	2.9 (2.2, 3.6) (a-c, h)	0.003	0.11
Sperm total count (million)	143 (70–293)	128 (80, 192) (a, b, d-f, i)	147 (36–348)	112 (67, 175) (a, b, d-f, i)	98 (42–187)	71 (38, 120) (a, b, d-f, i)	96 (48–241)	79 (35, 150) (a, b, d-f, i)	0.009	0.002
Normal morphology sperm (%)	5.5 (3.0–9.0)	3.4 (1.6, 6.0) (a-l)	5.5 (3.0–8.5)	3.4 (1.6, 6.3) (a-l)	5.0 (3.0–7.5)	2.9 (1.2, 5.6) (a-l)	6.0 (3.0–10.0)	3.9 (1.6, 7.7) (a-l)	0.42	0.45
Motile sperm (%)	71 (63–77)	83 (60, 94) (a-g, i-o)	69 (61–76)	83 (60, 94) (a-g, i-o)	70 (61–78)	82 (57, 94) (a-g, i-o)	66 (60–81)	83 (60, 95) (a-g, i-o)	0.71	0.97
Testicular volume (ml)	14.3 (10.0–17.5)	12.9 (10.7, 15.3) (a-e, g, h, m)	12.0 (10.0–15.0)	12.0 (9.9, 14.5) (a-e, g, h, m)	12.8 (10.0–17.5)	13.4 (10.7, 16.4) (a-e, g, h, m)	12.0 (11.0–15.0)	11.9 (8.6, 15.9) (a-e, g, h, m)	0.66	0.62

* The following characteristics were chosen as the referent in the models: between 48 hours and 5 days of abstinence, no daily smoking, sampling between October and March, no history of diseases in the reproductive organs, normal weight (body mass index between 20 and 25 kg/m^2), no weekly alcohol consumption, maternal drinking <4 cups (<0.95 liter) of coffee per day during pregnancy, birth weight 3,500 g, no maternal alcohol consumption during pregnancy, maternal age 25 years, no daily coffee consumption, parents' combined socioeconomic group I, no spillage at sampling, and no fever within the last 3 months.

† The back-transformed mean is adjusted for the following: abstinence time (a), current smoking (b), season (c), diseases in the reproductive organs (d), body mass index (e), alcohol (f), maternal coffee consumption during pregnancy (g), birth weight (h), maternal alcohol consumption during pregnancy (i), maternal age (j), coffee (k), socioeconomic group (l), spillage at sampling (m), recent fever (n), and minutes from ejaculation to start of analysis (o).

‡ Tested by Spearman's rank correlation test (medians) and generalized multiple linear regression (means).

§ CI, confidence interval.

TABLE 3. Odds ratio for oligospermia* among 347 young Danish men in relation to maternal smoking during pregnancy, 2005–2006†

Maternal smoking during pregnancy (cigarettes/day)	No. of cases	%	Crude odds ratio	95% confidence interval	Adjusted odds ratio‡	95% confidence interval
Nonsmoker ($n = 98$)	15	15	Referent		Referent	
1–9 ($n = 111$)	28	25	1.87	0.93, 3.75	1.88	0.89, 3.97
10–19 ($n = 106$)	25	24	1.71	0.84, 3.47	1.71	0.77, 3.83
>19 ($n = 28$)	9	32	2.62	1.00, 6.88	2.16	0.68, 6.87

* Sperm cell concentration >0 and <20 million/ml.

† The following characteristics were chosen as the referent in the models: between 48 hours and 5 days of abstinence, no daily smoking, sampling between October and March, no history of diseases in the reproductive organs, normal weight (body mass index between 20 and 25 kg/m^2), no weekly alcohol consumption, and maternal coffee consumption during pregnancy <4 cups/day.

‡ Adjusted for abstinence time, current smoking, season, diseases in the reproductive organs, body mass index, alcohol, and maternal coffee consumption during pregnancy.

sperm count with increased maternal smoking during pregnancy remained after adjustment ($p_{trend} = 0.002$).

Men exposed to heavy smoking in fetal life had approximately 19 percent lower mean semen volume ($p = 0.04$), 38 percent lower mean total sperm count ($p = 0.11$), and moderately lower (17 percent) mean sperm concentration ($p = 0.47$) compared with the nonexposed.

We found no associations between maternal smoking during pregnancy and testicular volume or percentages of motile and morphologically normal sperm (table 2).

We used the mothers' levels of smoking in the 36th week of gestation as the exposure. Of the 347 mothers, 205 (59 percent) reported no change in smoking habits during pregnancy, while 135 (39 percent) reported that they had changed to low-nicotine-content cigarettes, smoked a smaller part of the cigarettes, and/or tried to quit or reduce smoking. Information was missing for seven mothers. We repeated the analysis, including only men whose mothers had not changed smoking habits during pregnancy, and the magnitude of the effect of maternal smoking on the outcome variables was essentially the same as before (data not shown). We also based the analytical model on the ''change-in-estimate'' principle, and the findings were virtually unaffected.

We repeated the analysis using paternal (husband's) smoking during pregnancy as the explanatory variable, controlling for maternal smoking during pregnancy and the other covariates included in the former models. We found no dose-response relation between paternal smoking during pregnancy and the outcome variables (data not shown). Adjustment for paternal smoking in the multiple regression analysis did not change any of the associations for maternal smoking (data not shown).

Four (1.2 percent) men had azoospermia, and 77 (22.2 percent) men had oligospermia (excluding azoospermia). The crude and adjusted odds ratios for oligospermia in relation to prenatal tobacco exposure are shown table 3. There was a tendency toward an increased odds ratio for oligospermia with increased maternal smoking during pregnancy; the adjusted odds ratio was 1.26 (95 percent confidence interval (CI): 0.91, 1.75) for each increment. The adjusted odds ratio for oligospermia among men exposed to heavy smoking in fetal life was 2.16 (95 percent CI: 0.68, 6.87) compared with unexposed men.

DISCUSSION

To the best of our knowledge, this is the first population-based follow-up study based on prospectively collected data on prenatal tobacco exposure addressing semen quality in early adult life. Prenatal exposure to maternal smoking was associated with low semen volume and low total sperm count, and there was a tendency toward a lower sperm concentration for men exposed to heavy tobacco smoking in fetal life. The most exposed group had an adjusted odds ratio for oligospermia of 2.16 (95 percent CI: 0.68, 6.87) compared with unexposed men. We found no effect of prenatal tobacco exposure on sperm motility or morphology.

Although all these findings may be spurious or due to chance, it is not unexpected that exposures during the time period of testes development may impair spermatozoa production 20 years later in life. Cotinine (the major nicotine metabolite) crosses the placenta easily and is found in both amniotic fluid and fetal serum (11). In adult men, cotinine is found in seminal plasma in concentrations similar to those in serum (12). It has been suggested that cotinine also has an ability to cross the blood-testis barrier, and one or more of the chemical components in tobacco smoke may have a direct toxic effect on the germinative epithelium in the fetal testes (13). An effect via the fetal hypothalamic-pituitary-gonadal system is possible (14). The decrease in both semen volume and sperm concentration (that contributed to the decrease in total sperm count) may suggest an effect on fetal Sertoli cell development (15), as well as on the accessory sex glands. We did not find an association between tobacco exposure and testicular volume, perhaps because data on testicular volume were measured with some uncertainty and only available for 59 percent. In a study of mice

prenatally exposed to polycyclic aromatic hydrocarbons, the major toxic compounds in cigarette smoke, an inverse dose-response relation between exposure and testicular weight in addition to atrophy of seminiferous tubules and altered spermatogenesis was observed (16).

Storgaard et al. (2) found a lower sperm concentration and total sperm count only in men exposed to more than 10 cigarettes/day in fetal life compared with unexposed men. Our results are also compatible with a threshold for effect around 10 cigarettes/day rather than a dose-response effect. Jensen et al. (4) had information on only smoking or nonsmoking. In both studies, the magnitudes of the observed associations were somewhat larger than we found, and no effect on semen volume was found, but smoking was not the primary hypothesis in their studies. As in our study, no effect on sperm morphology was found.

Strengths of our study include use of prospectively collected data and the opportunity to sample sons exposed to maternal smoking of 20 or more cigarettes/day during pregnancy, although this group included only 28 men. In order to include this exposure experience, we sampled these men from a larger geographic recruitment area than the other groups, and we do not expect this to cause bias. No association between population density and semen quality has been observed in a study including 430 men from all over Denmark (J. P. Bonde, Department of Occupational Medicine, Aarhus University Hospital, personal communication, 2006), and living conditions and population density do not vary much in the areas we included in the study. In the recruitment procedure, priority was given to men whose mothers had answered a follow-up questionnaire in 2002, and we find it unlikely that this could cause bias. Within each exposure group, no differences in birth weight or sperm concentration were observed between men whose mothers answered the questionnaire in 2002 and men whose mothers did not respond to the questionnaire.

Our participation rate (49 percent) was rather high for semen quality studies but not high enough to exclude selection bias. We find it, however, unlikely that the participants, due to their age and lack of reproductive experience, would be able to self-select themselves for the study in a way that causes selection bias. The source population is young, most had no reproductive experience, and they were not aware of the exact hypothesis to be evaluated. If selection bias explains our results, prenatally exposed men with poor semen quality must have a higher participation rate. We compared sperm concentration among participants who responded to the first invitation letter (fast responders, 62 percent) with the sperm concentration among participants who responded to the reminder (slow responders, 38 percent) and found a tendency toward lower sperm concentrations among slow responders. This tendency was strongest for the exposed, but most of the difference disappeared, however, after adjustment. If nonresponders are more similar to the slow responders than to the fast responders concerning semen quality, the direction of the selection bias, if it exists, would be toward the no-effect level. Comparing participants and nonparticipants who filled in the small questionnaire on health ($n = 82$), we found no differences in the proportion of men with diseases of the reproductive organs. More importantly, the proportions did not differ between exposed and nonexposed nonparticipants. The proportion of smokers was lower among nonparticipants compared with participants (odds ratio = 0.32, 95 percent CI: 0.17, 0.58), but the difference was not related to exposure. Use of the other data source on nonparticipants ($n = 134$) showed the same tendencies. We do not expect loss to follow-up to have caused the associations that we found, but we have no data to rule this out.

Our data on maternal smoking during pregnancy were based on self-reports, but we believe that the data are quite accurate. Smoking during pregnancy was socially accepted at that time period in Denmark, and although some smokers may have denied smoking, it is unlikely that nonsmokers would claim to be smokers. Moreover, we found the expected smoking effect on birth weight: 138 (95 percent CI: 81, 195)-g lower mean birth weight per increase in exposure level.

In the analysis, we controlled for abstinence time and a number of other potential confounders. We were also able to control for maternal alcohol and coffee consumption during pregnancy and parents' socioeconomic group, which earlier studies did not adjust for, but in spite of this we cannot rule out confounding by other unknown factors.

In our study, 23 percent had sperm concentrations below the World Health Organization referent level of 20 million/ml, and the risk for oligospermia increased with increased maternal smoking during pregnancy. The median sperm concentration among all participants was 40 (25th–75th percentiles: 21–86) million/ml and 33 (25th–75th percentiles: 12, 75) million/ml among men exposed to 20 or more cigarettes/day in fetal life. Because fecundity increases with sperm concentrations up to approximately 40 million/ml (17), this may have consequences for some of the young men when they want to father a child. One may question the sexual maturity of our participants, but in a study of 158 men with a mean age of 19.1 years at baseline and followed quarterly for 4 years, no change in sperm concentration, total sperm count, or percentage of morphologically normal sperm was observed (18). We cannot, however, rule out that the association we see can be caused by a delayed puberty induced by prenatal tobacco exposure.

In conclusion, we found an inverse association between exposure to maternal smoking in fetal life and semen volume, sperm concentration, and total sperm count in adult life. The associations were not strong, and only total sperm count remained statistically significant after controlling for confounders. Sperm motility and sperm morphology were not affected. If these associations are causal, prenatal smoking exposure may explain at least part of the secular changes in semen quality over time and between populations.

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