Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index

Trine L. Ravnborg¹, Tina K. Jensen¹, Anna-Maria Andersson¹, Jorma Toppari², Niels E. Skakkebæk¹, and Niels Jørgensen¹,*
¹University Department of Growth and Reproduction, Rigshospitalet, Section 5064, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
²Department of Physiology and Paediatrics, University of Turku, FI-20520 Turku, Finland
*Correspondence address. Tel.: +4535455085; Fax: +4535456054; E-mail: niels.joergensen@rh.regionh.dk
Submitted on August 31, 2010; resubmitted on December 21, 2010; accepted on January 10, 2011

Background: Exposure to tobacco smoking prenatally is a risk factor for reduced semen quality, but whether the exposure has adverse effects on reproductive hormones, pubertal development or adult BMI remain largely unexplored. The aim of this study was to investigate the associations between these factors while controlling for the effects of current smoking in young adulthood.

Methods: This cross-sectional study (1996–2006) included 3486 Danish men (median age: 19 years), participating in a semen-quality study. Data were obtained from questionnaires, physical examinations, semen analyses and assessments of reproductive hormones. The main outcome measures were markers of pubertal onset, BMI, reproductive hormones and semen variables.

Results: Maternal smoking during pregnancy was associated with earlier onset of puberty (e.g. early pubic hair development in 25.2 versus 18.9% of unexposed subjects), lower final adult height (median: 1.80 versus 1.82 cm), higher BMI (22.9 versus 22.4), smaller testicles (14.0 versus 14.5 ml), lower total sperm counts (119 versus 150 million), reduced spermatogenesis-related hormones (e.g. inhibin-B/FSH 66 versus 73 pg/mU) and higher calculated free testosterone (free-T, 2.38 versus 2.33 nmol/l). If not exposed prenatally, men’s own smoking was associated with increased total testosterone but unchanged free-T. For smokers who had been exposed prenatally, total testosterone was increased but free-T was reduced (2.30 versus 2.38 nmol/l, P = 0.003) due to higher levels of sex hormone-binding globulin.

Conclusions: Prenatal exposure to tobacco may lead to faster pubertal development possibly caused by a higher free-T, and to higher adult BMI and impairment of testicular function. The findings may not be clinical relevant for the individual but are of public health importance, and add to the knowledge of effects of tobacco smoking.

Key words: tobacco smoking / prenatal exposure / reproductive hormones / semen quality / male reproduction

Introduction

Prenatal exposure of human male fetuses to maternal tobacco smoking has been associated with reduced testis size and lower semen quality in adulthood. Lower sperm concentrations, total sperm counts and numbers of motile and morphologically normal sperm have been found in exposed men (Jensen et al., 2004; Paasch et al., 2008), and a negative dose–response has been indicated (Storgaard et al., 2003; Jensen et al., 2005; Ramlau-Hansen et al., 2007). Supporting the suggested adverse effects, in utero tobacco exposures have recently been associated with reduced numbers of germ and somatic cells in embryonic male and female gonads (Mamsen et al., 2010). Animal studies have illustrated that in utero exposure to anti-androgenic agents may reduce Sertoli cell numbers that are the major factor determining sperm count in an individual (Scott et al., 2007; Sharpe, 2010), but have also shown that a continued post-natal exposure may be needed to suppress a post-natal recovery (Auharek et al., 2010). This indicates that both pre- and
post-natal exposures are needed to affect Sertoli cell number and spermatogenesis permanently, at least if gross abnormalities are otherwise not present.

The reduced sperm counts in prenatally exposed men (Storgaard et al., 2003; Jensen et al., 2004; Jensen et al., 2005; Ramlau-Hansen et al., 2007; Paasch et al., 2008) have not been clearly associated with altered levels of spermatogenesis-related hormones in adulthood (Ramlau-Hansen et al., 2007; Ramlau-Hansen et al., 2008; Kerkhof et al., 2009), as would be expected. Furthermore, limited research has been performed regarding the influence of in utero exposure to tobacco smoking on the adult testosterone-related endocrine functions, but a higher calculated free testosterone (free-T) level has been reported as a consequence (Kerkhof et al., 2009).

The previously mentioned epidemiological studies may have had limited power to detect subtle endocrine effects of prenatal tobacco exposure. We therefore studied independent effects of prenatal and current exposures to tobacco smoking on measurable markers of testicular function and self-reported markers of puberty in a cross-sectional study of more than 3000 young Danish men from the general population to provide further information about effect of prenatal tobacco exposure while taking into account current smoking.

Materials and Methods

Population

From 1996 to 2006 4862 Danish men from the Copenhagen area participated in a semen-quality study (participation rate: 24%). Information of in utero exposure to maternal smoking, own current smoking status and reproductive hormone levels were available for 3486 (mean/median age: 19.4 /19.0 years). Some results regarding the effect of maternal smoking on semen quality for 773 of the men examined from June 1996 to March 1998 have previously been published (Jensen et al., 2004).

Details of the general study design have been published (Jørgensen et al., 2002). Because of the military drafting system in Denmark 18-year-old men are required to undergo a compulsory examination to determine their fitness for service. Some men postpone their service to continue their education and are therefore not examined until they have completed their education. Since September 1996 we have approached the men when they appeared for this compulsory investigation and invited them to participate in a study of semen quality. Further eligibility criteria were that the men lived in the Copenhagen area and that they and their mothers were born and raised in Denmark. Men were eligible irrespective of their fitness for military service. The participants answered a questionnaire, underwent a physical examination, provided a semen sample and had a blood sample drawn. They received €65 euros for their participation. The Science Ethical Committee for the Copenhagen and Frederiksberg municipalities approved the study, and all participants gave their informed consent.

Questionnaire

The participants completed the questionnaire, which was sent to them prior to their attendance in the study, in collaboration with their mother and/or father. The questionnaire included information on whether the men were tobacco smokers themselves, their usual number of cigarettes smoked daily, if mothers and fathers smoked while mothers were pregnant (yes/no), if anyone smoked at home during their childhood and the men’s birthweight and length. They also answered whether they had experienced puberty changes (voice break, pubic hair development or growth of penis) at the same time, earlier or later than friends of similar ages. Questions regarding previous and/or current diseases including genital diseases such as cryptorchidism, testicular torsion, hydrocele, varicocele, orchitis, epididymitis, chlamydia and gonorrhoea were also included. Information on alcohol intake during the week before participation in the study was also provided as intake of bottles of beers (0.33 l), glasses of wine (units) and number of strong alcholic drinks (2 cl).

Physical examination

Physical examinations, performed the day the men delivered their semen sample, included assessment of body weight and height (using a Harpenden stadiometer), size of the testicles determined by ultrasound and the presence of varicocele, hydrocele or any other genital malformations.

Semen analysis

Each man provided a semen sample by masturbation in a room close to the laboratory. The ejaculation abstinence period prior to sampling was recorded. The sample was collected in a plastic container and kept at 37°C until analysis. Semen volume was estimated by weighing the collection tube, and the percentage of motile spermatozoa was determined. Sperm concentration was assessed using a Burker-Türk haemocytometer, and sperm morphology was assessed according to strict criteria. Morphology slides were assessed for only 1897 men (54.4%) due to lack of resources, however, the slides were randomly selected. Total sperm count was calculated as semen volume x sperm concentration. Details of the methods have previously been described (Jørgensen et al., 1997, 2002).

Hormone analyses

Following semen delivery, a venous blood sample was taken. The sample was centrifuged, and the serum was separated and kept frozen until analysis. Serum levels of follicle-stimulating hormone (FSH), luteinising hormone (LH), sex hormone-binding globulin (SHBG) and testosterone were determined using a time-resolved fluoroimmunoassay (Delfia, Wallac, Turku, Finland), estradiol was determined by radioimmunoassay (Pantex, Santa Monica, USA) and inhibin-B was determined by a specific two-sided enzyme immunometric assay. The intra- and inter-assay coefficients of variation for measurement of FSH and LH were 3 and 4.5% respectively. CVs for both testosterone and SHBG were <8 and <5%, respectively. For estradiol the intra-and inter-assay CVs were 7.5% and 13% and for inhibin-B the CVs were 15% and 18%. Estradiol was only assessed for samples collected in 2003–2006. The free androgen index (FAI) was calculated as (total testosterone × 100)/SHBG and free-T was calculated from total testosterone and SHBG using a fixed albumin value according to Vermeulen et al. (1999).

Statistical analysis

The men were stratified into four groups according to their exposure to maternal smoking during pregnancy (yes–no) and their own smoking status (yes–no) (Table I). For continuous variables, median and 5–95 percentiles were calculated. Mean and standard deviations were also calculated but only reported in selected cases (e.g. birth length, explanatory comments to Table I). Frequencies were calculated for the categorical variables. Between-group differences of these basic descriptions were all tested by chi-square test (called ‘P-value (1)’ in Table I). Furthermore, for the continuous variables the between-group differences for exposure to maternal smoking were tested by regression analyses while controlling for the men’s smoking status (called ‘P-value (2)’ in the table) and vice versa (called ‘P-value (3)’ in the table). For the categorical variables, a similar approach to test the between-group differences for exposure to
Table I Characteristics of 3486 young Danish men according to their smoking habits and exposure to maternal tobacco smoking during pregnancy.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother smoked during pregnancy</td>
<td>Men smokers</td>
<td>16% (n = 562)</td>
<td>558</td>
<td>19.0 (18.4–21.2)</td>
<td>814</td>
<td>19.0 (18.4–21.8)</td>
<td>764</td>
<td>18.9 (18.4–21.6)</td>
<td>1324</td>
<td>18.9 (18.4–21.9)</td>
</tr>
<tr>
<td>Mother did not smoke during pregnancy</td>
<td>Men non-smokers</td>
<td>24% (n = 823)</td>
<td>648</td>
<td>3.35 (2.29–4.25)</td>
<td>623</td>
<td>5.0 (4.7–5.5)</td>
<td>496</td>
<td>5.0 (4.8–5.7)</td>
<td>1244</td>
<td>5.0 (4.8–5.7)</td>
</tr>
<tr>
<td>Men smokers</td>
<td>Men non-smokers</td>
<td>22% (n = 769)</td>
<td>764</td>
<td>19.0 (18.4–21.8)</td>
<td>767</td>
<td>19.1 (18.7–21.9)</td>
<td>767</td>
<td>18.9 (18.4–21.9)</td>
<td>1244</td>
<td>18.9 (18.4–21.9)</td>
</tr>
<tr>
<td>Men non-smokers</td>
<td>Men non-smokers</td>
<td>38% (n = 1332)</td>
<td>1324</td>
<td>18.9 (18.4–21.9)</td>
<td>1327</td>
<td>18.9 (18.4–21.9)</td>
<td>1327</td>
<td>18.9 (18.4–21.9)</td>
<td>1327</td>
<td>18.9 (18.4–21.9)</td>
</tr>
<tr>
<td>Difference between four groups</td>
<td>Effect of maternal smoking</td>
<td>Effect of men's smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>No.</td>
<td>Frequency (%)</td>
<td>No.</td>
<td>Frequency (%)</td>
<td>No.</td>
<td>Frequency (%)</td>
<td>No.</td>
<td>Frequency (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parents helped answering questionnaire</td>
<td>515</td>
<td>74.8</td>
<td>746</td>
<td>84.9</td>
<td>699</td>
<td>73.8</td>
<td>1191</td>
<td>79.2</td>
<td>&lt;0.0005</td>
<td>0.01</td>
</tr>
<tr>
<td>Father smoked during mothers pregnancy</td>
<td>354</td>
<td>75.1</td>
<td>530</td>
<td>68.5</td>
<td>490</td>
<td>44.5</td>
<td>856</td>
<td>33.8</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Passive smoking during childhood</td>
<td>350</td>
<td>97.7</td>
<td>527</td>
<td>97.3</td>
<td>481</td>
<td>53.8</td>
<td>845</td>
<td>41.4</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Cryptorchidism</td>
<td>561</td>
<td>11.8</td>
<td>819</td>
<td>12.6</td>
<td>766</td>
<td>10.3</td>
<td>1327</td>
<td>9.3</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Early voice break</td>
<td>341</td>
<td>23.8</td>
<td>823</td>
<td>18.3</td>
<td>475</td>
<td>19.8</td>
<td>839</td>
<td>14.9</td>
<td>0.003</td>
<td>0.03</td>
</tr>
<tr>
<td>Early growth of penis</td>
<td>343</td>
<td>17.5</td>
<td>517</td>
<td>13.9</td>
<td>482</td>
<td>14.5</td>
<td>839</td>
<td>11.2</td>
<td>0.03</td>
<td>0.006</td>
</tr>
<tr>
<td>Event</td>
<td>Mean (SD) Group 1</td>
<td>Mean (SD) Group 2</td>
<td>Mean (SD) Group 3</td>
<td>Mean (SD) Group 4</td>
<td>P-value (1)</td>
<td>P-value (2)</td>
<td>P-value (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early pubic hair development&lt;sup&gt;h&lt;/sup&gt;</td>
<td>337 30.0</td>
<td>511 25.2</td>
<td>475 23.2</td>
<td>830 18.9</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had sexually transmitted diseases&lt;sup&gt;i&lt;/sup&gt;</td>
<td>471 4.0</td>
<td>677 3.8</td>
<td>608 4.3</td>
<td>1102 2.2</td>
<td>0.06</td>
<td>0.2</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has caused a pregnancy&lt;sup&gt;j&lt;/sup&gt;</td>
<td>562 12.3</td>
<td>821 4.8</td>
<td>769 8.5</td>
<td>1331 2.8</td>
<td>&lt;0.0005</td>
<td>0.001</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has experienced fertility problems&lt;sup&gt;k&lt;/sup&gt;</td>
<td>477 2.3</td>
<td>689 0.7</td>
<td>631 1.1</td>
<td>1115 0.4</td>
<td>0.002</td>
<td>0.09</td>
<td>0.006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taken medicine&lt;sup&gt;l&lt;/sup&gt;</td>
<td>562 13.9</td>
<td>823 17.9</td>
<td>769 14.3</td>
<td>1332 17.6</td>
<td>0.05</td>
<td>0.9</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;21 units of alcohol recent week&lt;sup&gt;c&lt;/sup&gt;</td>
<td>457 26.9</td>
<td>653 16.8</td>
<td>606 28.4</td>
<td>1073 15.9</td>
<td>&lt;0.0005</td>
<td>0.9</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No alcohol recent week&lt;sup&gt;c&lt;/sup&gt;</td>
<td>457 11.4</td>
<td>653 17.5</td>
<td>606 9.7</td>
<td>1073 19.2</td>
<td>&lt;0.0005</td>
<td>0.8</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No., number of men for which information was available; (5–95), 5–95th percentile.  

P-value (1): Chi-square test comparing all four groups of men.  

P-value (2): Comparing effects of maternal smoking during pregnancy. For continuous variables based on regression analyses controlled for men's own smoking status. For categorical variables based on Mantel–Haenszel statistics comparing effects of maternal smoking during pregnancy controlling for the effect of the men's own smoking status.  

P-value (3): Comparing effect of men's own smoking status. For continuous variables based on regression analyses controlled for effect of maternal smoking during pregnancy. For categorical variables based on Mantel–Haenszel statistics comparing effects of men's own smoking status controlling for the effect of maternal smoking during pregnancy.  

<sup>a</sup> Mean (standard deviation) for the four groups were (cm): 52.1 (2.7), 51.2 (3.1), 52.1 (2.9) and 51.3 (3.3).  

<sup>b</sup> Mean of two testicles.  

<sup>c</sup> Alcohol intake was the sum of bottles of beer, glasses of wine and number of strong alcoholic drinks the recent week prior to study participation.  

<sup>d</sup> Exposed to passive smoking during childhood by a member of household.  

<sup>e</sup> Born with cryptorchidism irrespective of later spontaneous descent, treatment or still cryptorchid.  

<sup>f</sup> Time of voice break in comparison to children of the same age reported as earlier, same time or later.  

<sup>g</sup> Time of penis growth in comparison to children of the same age reported as earlier, same time or later.  

<sup>h</sup> Time of pubic hair development in comparison to children of the same age reported as earlier, same time or later.  

<sup>i</sup> Previously diagnosed with chlamydia or gonorrhoea.  

<sup>j</sup> Ever caused a pregnancy.  

<sup>k</sup> Ever had regular intercourse without use of contraception for at least 1 year, without partner became pregnant.  

<sup>l</sup> Taken any medication recent 3 months prior to participation in the study. For 57% it was over-the-counter painkillers, antibiotics or against allergy.
maternal smoking was taken using Mantel–Haenszel statistics while controlling for equal effects in the two groups of smoking men, and vice versa.

The main outcome variables included the assessed hormone and semen variables, and the between-group differences were tested by multiple linear regression. To obtain normality of the residuals, reproductive hormone levels, semen volumes, sperm concentrations and total sperm counts were transformed by the natural logarithm. The percentages of motile spermatozoa were logit-transformed, whereas percentages of morphologically normal spermatozoa entered the model untransformed. Hormone levels, semen volumes, sperm concentrations and total sperm counts were transformed by the natural logarithm. The percentages of motile spermatozoa were logit-transformed, whereas percentages of morphologically normal spermatozoa entered the model untransformed.

Regression models as covariate. For all semen variables, history of genital disease and age was also controlled for.

Linear regressions were also done to give calculated expected levels for a 19-year-old man, without any genital diseases in his self-reported history and having a BMI of 22. Additional covariates were adjusted for: (i) semen volume, sperm concentration and total sperm count to represent an ejaculation abstinence period of 96 h, (ii) motility to represent assessment duration of 30 min from ejaculation to assessment.

The above-mentioned confounders are all well known to affect semen and hormonal variables. The mentioned analyses were made with further additional potential confounders such as season of investigation, birthweight, exposure to passive smoking in childhood, coffee and food consumption (use of butter on bread, frequency of eating fast food, vegetables and fruit), use of medicine in the 3 months before participation in the study, and self-reported alcohol intake in the recent week. Inclusion of these additional variables did not change the estimates, and were therefore not included in the final statistical models. Analyses were also

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mother smoked during pregnancy</th>
<th>Mother did not smoke during pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men smokers</td>
<td>Unadjusted median (5–95)</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>No</td>
<td>818</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>558</td>
</tr>
<tr>
<td>Total sperm counts (mill)</td>
<td>No</td>
<td>818</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>558</td>
</tr>
<tr>
<td>Morphological normal forms (%)</td>
<td>No</td>
<td>452</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>316</td>
</tr>
<tr>
<td>Motile spermatozoa (%)</td>
<td>No</td>
<td>816</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>557</td>
</tr>
<tr>
<td>Inhibin-B (pg/ml)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>561</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Inhibin-B/FSH</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>561</td>
</tr>
</tbody>
</table>

No., number of men in each stratified category. Note that morphology was not assessed for all men; unadjusted median (5–95): unadjusted median and 5–95th percentiles; adjusted median (95% CI): adjusted medians and 95% confidence interval. Hormones adjusted to give estimates for a 19 year old man, having BMI of 22, no genital diseases and blood sample drawn at 8 a.m. Semen volume, sperm concentration and total counts adjusted to an ejaculation abstinence period of 96 h. Motility adjusted to a duration of 30 min from ejaculation to assessment.

P-value (1): P-values comparing men exposed to maternal smoking in utero versus non-exposed men.
P-value (2): P-values comparing smoking men versus non-smokers.
of the men, 40% (n = 1385) had been exposed to maternal smoking prenatally, and they were more often smokers than the non-exposed men (41 versus 37%, P = 0.02). For 78.6% of the participants, one or both parents had contributed to the answering of the questionnaire. With fewer among the smokers than the non-smokers. The exposed men had lower birthweights, birth lengths, lower final adult height, higher BMI and smaller testicles (as assessed by ultrasound; Table I, P-value (2)'). The median testis size assessed by palpation using a Prader orchidometer was 20 ml (5–95th percentile: 12.5–27.5 ml) for the combined group of men, however, there were slightly higher mean values for those who had not been exposed prenatally (20.4 and 20.3 ml, P = 0.9 for smoking and non-smoking men, respectively, and 19.8 and 19.9 ml, P = 0.002 for in utero exposure). Most men (99.4%) had a mature adult pubic hair distribution (Tanner stage 5), with 0.6% at Tanner stage 4 (P = 0.4 for the difference between the four subgroups of men).

The in utero exposed men had more frequently had cryptorchidism, self-reported signs of early puberty (voice break, growth of penis and pubic hair development), and had more often been exposed to passive smoking at home during childhood. Six per cent of the participants had caused a pregnancy, most among men that were prenatally exposed and smokers themselves (12.3%) and fewest among men that had

### Results

#### Basic characteristics

Of the men, 40% (n = 1385) had been exposed to maternal smoking prenatally, and they were more often smokers than the non-exposed men (41 versus 37%, P = 0.02). For 78.6% of the participants, one or both parents had contributed to the answering of the questionnaire. With fewer among the smokers than the non-smokers. The exposed men had lower birthweights, birth lengths, lower final adult height, higher BMI and smaller testicles (as assessed by ultrasound; Table I, P-value (2)'). The median testis size assessed by palpation using a Prader orchidometer was 20 ml (5–95th percentile: 12.5–27.5 ml) for the combined group of men, however, there were slightly higher mean values for those who had not been exposed prenatally (20.4 and 20.3 ml, P = 0.9 for smoking and non-smoking men, respectively, and 19.8 and 19.9 ml, P = 0.002 for in utero exposure). Most men (99.4%) had a mature adult pubic hair distribution (Tanner stage 5), with 0.6% at Tanner stage 4 (P = 0.4 for the difference between the four subgroups of men).

The in utero exposed men had more frequently had cryptorchidism, self-reported signs of early puberty (voice break, growth of penis and pubic hair development), and had more often been exposed to passive smoking at home during childhood. Six per cent of the participants had caused a pregnancy, most among men that were prenatally exposed and smokers themselves (12.3%) and fewest among men that had

### Table III Leydig cell-related hormones in 3486 young Danish men stratified according to their exposure in utero to maternal smoking and according to their own current smoking status.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Mother smoked during pregnancy</th>
<th>Mother did not smoke during pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men smokers</td>
<td>Unadjusted median (5–95)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Free-T (nmol/l)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>LH (U/l)</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Total testosterone/LH</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Free-T/LH</td>
<td>No</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>562</td>
</tr>
<tr>
<td>Estradiol (nmol/l)*</td>
<td>No</td>
<td>307</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>212</td>
</tr>
</tbody>
</table>

No. = number of men in each stratified category. Note that estradiol was not measured in all men; unadjusted median (5–95): unadjusted median and 5–95th percentiles; adjusted median (95% CI): adjusted medians and 95% confidence interval. Results adjusted to give estimates for a 19 year old man, having BMI of 22, no previous or current genital diseases and blood sample drawn at 8 a.m.

*P*-value (1): P-values comparing smoking men versus non-smoking men.

P-value (2): P-values comparing smoking men versus non-smokers.

*Estradiol in 519 men exposed to maternal smoking in utero was 76 (48–116) nmol/l versus 71 (46–114) in 883 non-exposed, and in this combined groups P-value (1) is 0.02.

---

repeated for the 78.6% of participants who had help from their parents to answer the questions.

Thirdly, the same analyses as described above were performed categorizing the participating men into non-smokers, light smokers (1–10 cigarettes daily) or heavy smokers (more than 10 cigarettes daily) rather than into smokers and non-smokers. This grouping did not provide

Thein utero exposed men had more frequently had cryptorchidism, self-reported signs of early puberty (voice break, growth of penis and pubic hair development), and had more often been exposed to passive smoking at home during childhood. Six per cent of the participants had caused a pregnancy, most among men that were prenatally exposed and smokers themselves (12.3%) and fewest among men that had

---

**Tobacco and male reproductive hormones**

1005
Men’s own tobacco smoking was associated with lower adult weight, lower BMI and higher intake of alcohol (Table I, \(P\)-value (3)). Smokers also tended to have lower final adult height \((P = 0.05, \text{controlled for maternal smoking})\), and to have had signs of early puberty more frequently \((P\)-values = 0.02–0.06).

**Spermatogenesis-related variables**

Prenatal exposure to maternal smoking was associated with decreased sperm concentrations and total sperm counts (Table II, \(P\)-value (1)), and morphologically normal spermatozoa were slightly but significantly fewer in prenatally exposed if they were non-smokers themselves (6.4 versus 6.7%). Semen volume and percentage of motile spermatozoa did not differ depending on prenatal tobacco exposure. Prenatal exposure was also associated with lower inhibin-B and inhibin-B/FSH, whereas FSH levels in adulthood were unchanged. Men’s own smoking status was not associated with changes in semen quality or reproductive hormones neither when categorized yes–no (Table II, \(P\)-value (2)) nor when categorized as non-smokers, light smokers or heavy smokers (not shown). Exposure to passive smoking at home during childhood was non-significantly \((P = 0.3–0.9)\) related to the spermatogenesis-related variables.

**Testosterone-related variables**

Prenatal exposure to maternal tobacco smoking was, in adulthood, associated with lower SHBG if the men were non-smokers but not with alterations in the level of total testosterone (Table III). Consequently, prenatally exposed men who were not smokers had significantly higher free-T.

Total testosterone levels were higher for smokers than non-smokers. Smoking men who had been exposed in utero also had higher levels of SHBG. Thus, the free-T for these men was reduced, whereas it remained unchanged for men whose mothers did not smoke during pregnancy. LH or the hormone ratios total testosterone/LH and free-T/LH did not differ between prenatally exposed and non-exposed men. Estradiol was only assessed for 519 prenatally exposed and 883 non-exposed men, and no statistical significant differences were detected in men exposed in utero. LH did not differ depending on the men’s own smoking status. Figure 2 summarizes the findings regarding total testosterone, SHBG, free-T and estradiol.

Exposure to passive smoking at home during childhood was not significantly \((P = 0.2–0.6)\) related to the testosterone-related variables described above.

**Discussion**

In this study of almost 3500 young Danish men, the independent effects of both the prenatal and current exposures to tobacco smoking were investigated on several reproductive and physical outcomes. Maternal smoking during pregnancy was associated with a reduction in both the endocrine and exocrine primary testicular capacity. In addition, in utero exposure to maternal smoking was associated with earlier puberty and slightly reduced final height and increased BMI, which may be interrelated.
Prenatal exposure to maternal tobacco smoking

The lower levels of inhibin-B and inhibin-B/FSH among men exposed to cigarette smoking prenatally have to our knowledge not been published before. Inhibin-B correlates with sperm counts (Jensen et al., 1997; Pierik et al., 1998; Andersson et al., 2004a,b; Meeker et al., 2007; Jørgensen et al., 2010), and the lower inhibin-B/FSH indicated a primary reduction in the testicular spermatogenic capacity. The lack of a compensatory increase in FSH could additionally indicate that the hypothalamo–pituitary function had also been adversely affected by maternal smoking during pregnancy.

Inhibin-B levels show a strong positive correlation with sperm counts at low levels (inhibin-B < 150 pg/ml) (Jørgensen et al., 2010). The median levels here were >150 pg/ml, which may explain why prenatally exposed men had sperm counts at ~80% of non-exposed, while the spermatogenesis-related hormone inhibin-B was at ~90%.

The reduced sperm concentration, reduced total sperm count and smaller testis size among prenatally exposed confirm previous findings (Storgaard et al., 2003; Jensen et al., 2004, 2005; Paasch et al., 2008). We did not have information to examine if a dose–response for prenatal exposure exists. However, another Danish study of 522 mother-son pairs observed such a dose–response between maternal smoking and low sperm counts in the sons (Jensen et al., 2005).

Maternal smoking during pregnancy was associated with increased free-T in adult sons, which partly is in line with results by Kerkhof et al. (2009). If this increase was apparent already from the early onset of puberty, it can be hypothesized that these men had experienced a more rapid pubertal development with earlier signs of puberty. Early puberty leads to lower final height because of a shortened period of childhood growth (Carel et al., 2009). In combination with the known adverse effect of maternal smoking on birthweight and length (Wilcox, 1993; England et al., 2001; Varvargou et al., 2009), which we also detected, this might explain their shorter final height. Furthermore, smoking men had shorter final height and lower weight the more they smoked themselves even when controlled for maternal smoking (data not shown). This indicated that both prenatal exposure and an early onset of smoking may reduce final height. The cause–effect relationship is, however, not clear, since early puberty predisposes to risk behaviours such as smoking (Golub et al., 2008). Previous studies have indicated a catch-up growth among prenatally tobacco exposed children, but the follow-up period was only until adolescence (Vik et al., 1996; Ong et al., 2000; Sowan and Stember, 2000).

Prenatal exposure to tobacco smoke has been associated with behavioural problems in school-age children (Ruckinger et al., 2010). In our study, current smokers had higher alcohol consumption and a tendency for increased risk of sexually transmitted diseases, which could be due to different norms of behaviour. However, the higher free-T, caused by maternal smoking during pregnancy, may lead to a rapid pubertal development, which predisposes to risk-taking behaviour (Golub et al., 2008), which may contribute to the higher proportion of smokers among prenatally exposed men.

The slightly increased free-T in the adult sons of women who smoked during pregnancy was not caused by changes in total testosterone but by a reduction in SHBG. The reduction was not explained by differences in BMI, which we corrected for statistically. We cannot clarify whether this altered SHBG level is a direct consequence of maternal smoking or an indirect effect via decreased thyroid hormone or increased prolactin levels, which both can reduce SHBG (Selby, 1990). An animal study showed maternal nicotine exposure during lactation to cause a neonatal thyroid dysfunction,
increased adiposity, hyperleptinemia and secondary hypothyroidism in adulthood (Oliveira et al., 2009). Thus, an altered thyroid hormone level in prenatally exposed men would fit with the SHBG levels and higher BMI that we detected. However, information about thyroid hormones was not available in this study.

We could not detect any increased morbidity in the prenatally exposed men despite their altered BMI (not shown), probably because they were still very young. However, this group of men may be at risk for a more pronounced increase in BMI, which would agree with the finding by Power and Jefferts (2002) who detected an odds ratio of 1.56 for obesity at 33 years if exposed to maternal smoking during pregnancy. This increase in BMI associated with maternal smoking secondly could lead to a higher risk of metabolic syndrome or other BMI-related diseases later in life.

It would be expected that the lower SHBG level in men exposed prenatally was associated with a lower total testosterone if the free-T (calculated free-T or FAI) was unchanged. However, total testosterone was unchanged and therefore a higher free-T was noticed. Despite the higher free-T for prenatally exposed men, LH was not changed. We hypothesize that the exposure to tobacco smoking in utero may cause a change in the set point of the hypothalamic–pituitary (HP) axis, making the HP axis less sensitive to increased testosterone or estradiol levels.

Tobacco smoking in adulthood

Several publications have shown that smokers have higher total testosterone than non-smokers (Trummer et al., 2002; Svartberg et al., 2003; Ramlau-Hansen et al., 2007; Svartberg and Jorde, 2007), which we also detected. The higher levels did not seem to reflect maternal smoking during pregnancy, but resulted from the men’s own smoking habits. However, when we corrected for SHBG, the free-T level in smokers was not increased, but actually showed a non-significant decreasing tendency. This suggests that the higher total testosterone in smokers is an adjustment to a higher SHBG to avoid a decrease in the free-T.

While in utero exposure to maternal smoking was associated with a reduction in SHBG, the men’s own smoking was associated with increased SHBG, which has also been shown in some other studies but not all (Svartberg and Jorde, 2007; Ramlau-Hansen et al., 2008; Kerkhof et al., 2009). Hyperthyroidism and hepatic cirrhosis in men are associated with high concentrations of SHBG (Selby, 1990). The influence of cigarette smoking in adulthood on thyroid function is controversial (Müller et al., 1995; Jorde and Sundsfjord, 2006; Asvold et al., 2007; Vanderver et al., 2007). We have no data to explain the higher SHBG or to determine whether prenatally exposed men had a slight change in thyroid or liver function. The higher SHBG could not be attributed to BMI, which we corrected for, or to recent alcohol intake.

When controlled for maternal smoking, men’s own smoking was not associated with altered semen quality or FSH and inhibin-B levels. Our study subjects were relatively young resulting in a short smoke exposure time, and it cannot be excluded that a longer smoking period could lead to alterations (Shen et al., 1997; Sepaniak et al., 2006).

Validity of results

The participation rate is often low in studies that require delivery of semen samples. Despite this the participation rate was 24% and thus higher than in most other population-based semen-quality studies (Jørgensen et al., 2001, 2002; Punab et al., 2002; Swan et al., 2003; Paasch et al., 2008). Still the question whether the obtained results are actually representative for the general population arises and a potential bias should be considered. Previously, we have detected that the levels of the reproductive hormones FSH, inhibin-B, LH and testosterone did not differ between a group of men who delivered semen samples and a larger group of men who only had a blood sample drawn (Andersen et al., 2000). This indicates that men who participate in a semen-quality study using our approach do not differ from non-participants when their testicular function are assessed according to hormonal levels, indicating that the participation rate may not hamper conclusions from the obtained results. In the invitation to the study the possible effects of tobacco smoking were not mentioned. In addition, our goal was to compare testicular function among men with different exposures to tobacco, and therefore it is of secondary importance if the men were actually representative of the general population. Furthermore, because the majority of our young men had no knowledge of their own fertility potential, this factor is unlikely to have affected their motivation to participate. Thus, fertility problems or attention to smoking-related problems have probably not been a motivating factor to participate. The financial compensation received for participating in the study seems unlikely to have led to the selection of men with reduced semen quality. In fact, if compensation had not been provided, it is most likely that men suffering from some kind of disease would be more interested in participating (in the hope of receiving advice) than men without diseases.

We regard the obtained information about mothers smoking during pregnancy as valid. We only included 3486 of the 4862 men whose information on smoking habits and maternal smoking during pregnancy was not available. The lower birthweight among men that were classified as prenatally exposed is an expected finding (Wilcox, 1993; England et al., 2001; Varvarigou et al., 2009), which also support the validity of the collected information. However, we did not obtain information on whether the mother, the father or both helped answering the questions, but most likely this information is equally valid whether provided by mother or father. We did not obtain information about how much the parents smoked or if they stopped during the pregnancy. Thus, we cannot provide any ‘dose–response’ calculations. Other studies have, however, detected an inverse dose–response with semen parameters (Storgaard et al., 2003; Jensen et al., 2005; Ramlau-Hansen et al., 2007; Mamsen et al., 2010), but did not find a lower threshold.

The information regarding observed pubertal changes were not based on a previous physical examination of the men but rather self-reported information about timing of pubertal events. Bias in relation to the reported associations between in utero smoking exposure and pubertal changes is only likely if in utero-exposed men with early puberty were oversampled. This is unlikely, since in utero exposure...
to smoking is not an established risk factor for pubertal timing. Since the information rely on self-reported events they cannot be regarded as precise, and the effects we have detected could actually be more pronounced. Precise effects can only be detected in a study which includes more sensitive markers of puberty.

The higher frequency of men who, at age 19, had caused a pregnancy among in utero exposed and smokers could be a result of their earlier pubertal development and a more ‘out-reacting’ behaviour. Despite the generally lower semen quality among the in utero exposed their average level was still compatible with an almost normal chance of achieving a pregnancy within one menstrual cycle (Bonde et al., 1998). Thus, the higher frequencies should in our mind not be interpreted as some paradoxical positive effect of in utero exposure to tobacco smoking.

In addition to controlling for in utero smoke exposure and smoking habits of the men, the results were controlled for other factors related to semen quality and hormones (e.g. ejaculation abstinence time, time to analysis of motility, hour of blood samples, BMI, genitalic diseases, etc.). Secondly, the same analyses were made with further potential confounders such as season, birthweight, exposure to passive smoking in childhood, usual coffee and food consumption, use of medicine during preceding 3 months and self-reported alcohol intake during preceding recent week. Including these potential confounders did not change the results. Exposure to passive smoking in childhood did not influence the outcomes, but it should be remembered that smoke exposure in childhood was only classified as numbers of smokers at home, whereas we did not have information of the exact magnitude and timing of exposure.

Almost all participants had an adult pubic hair distribution, normal adult testis size (Tanner, 1962) and normal adult level of the reproductive hormones (Andersson et al., 2004a,b). Previously we followed some of these men over a 4 year period and their semen quality did not improve (Carlsen et al., 2005). Thus, immaturity does not seem to be a confounding factor.

**Conclusion**

In utero exposures of males to maternal tobacco smoking was associated with earlier onset of puberty, shorter final body height, increased BMI, increased free-T, reduced semen quality reflecting a primary testicular impairment and possibly also an altered hypothalamo-pituitary function. Men’s own smoking lead to increased total serum testosterone to compensate for increased SHBG to keep the free-T level unaltered. This was only partly possible if the men had also been exposed prenatally. Our findings add knowledge of effects of tobacco smoking. They may not be of direct clinical importance for the individual but may be of public health importance. Based on the results, we suggest that research should focus on whether fetal exposure to tobacco may lead not only to a reduced reproductive potential but also to a higher risk of metabolic syndrome or other BMI-related diseases and increased risk of late onset hypogonadism.

**Ethical approval**

The Danish National Committee on Biomedical Research Ethics, Copenhagen Region, approved the research, and all young men gave written informed consent.

**Authors’ roles**


**Funding**

N.J. has received financial support from the Danish Agency for Science, Technology and Innovation (grant no. 271070678). The studies of the young men from the general Danish population have been supported by the European Union (contract QLK4-CT-2002-00603 and most recently FP7/2007–2013, DEER grant agreement no. 212844) and the Danish Ministry of Health and Prevention (to N.J. and N.E.S.). The funding organizations played no role in the design and conduct of the study, in collection, management, analysis and interpretation of the data; or in the presentation, review or approval of the manuscript.

**References**


Auharek SA, de Franca LR, McKinnell C, Jobling MS, Scott HM, Sharpe RM. Prenatal plus postnatal exposure to Di(n-Butyl) Phthalate and/or Flutamide markedly reduces final Sertoli cell number in the rat. *Endocrinology* 2010; 151:2868–2875.


