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ORIGINAL ARTICLE

Association between tobacco exposure and reproductive parameters in adolescent males

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Summary

Cigarette smoking is quite prevalent in the general population but our knowledge of its effect on male reproductive function is still very limited. Therefore, we investigated the impact of tobacco exposure on reproductive characteristics in young males. Military conscripts, 217 non-smokers and 85 smokers, with a median age of 18 years were enrolled. Physical examination and semen analysis, including measurement of accessory sex gland markers and reproductive hormone levels, were performed. Lifestyle-associated factors, including maternal smoking during pregnancy and snuffing, were recorded. Non-smokers had 49% higher total sperm number than smokers (95% CI 4.5–112%, $p = 0.01$). In addition, sperm concentration was 37% higher among non-smokers (95% CI −4% to 95%, $p = 0.08$). Serum levels of follicle-stimulating hormone (FSH) were 17% higher among non-smokers (95% CI 3–33%, $p = 0.02$), whereas no significant differences between smokers and non-smokers were found for inhibin B, testosterone, sex hormone binding globulin, luteinizing hormone and oestradiol. Those who smoked >10 cigarettes per day exhibited 37% lower (95% CI 10–69%, $p = 0.005$) FSH levels than those who smoked less. Maternal smoking during pregnancy had a negative impact on epididymal and seminal vesicle marker secretion. Smoking seems to impair sperm production and epididymal as well as accessory sex gland function and could be one of the factors contributing to regional differences in sperm parameters.

Introduction

Despite the high prevalence of cigarette smoking in the general population, our knowledge of its impact on male reproductive function is still very limited. In a meta-analysis, smoking was only found to have an adverse effect on sperm concentration and motility in healthy volunteers and sperm donors, but not in infertility patients (Vine *et al.*, 1994). However, generally the results of studies concerning the association between smoking and male reproductive parameters are contradictory. Some studies have shown a negative effect of smoking on semen parameters (Shen *et al.*, 1997; Stutz *et al.*, 2004; Pasqualotto *et al.*, 2006) whereas others could not demonstrate such an effect (Vogt *et al.*, 1986; Saaranen *et al.*, 1987; Lewin *et al.*, 1991; Pacifici *et al.*, 1993). Furthermore, it has not been clarified whether smoking has an impact on levels of male reproductive hormones. Some recent studies indicated an increase in serum levels of inhibin B and testosterone in smokers (Jensen *et al.*, 2005; Svartberg & Jorde, 2006) whereas such effect was not found by others (Andersen *et al.*, 1984; Vogt *et al.*, 1986).

To date, the majority of studies on the association between smoking and semen quality and accessory sex gland function are based on individuals recruited from infertility clinics (Vine *et al.*, 1994). However, infertile men might not be representative of the general population with respect to susceptibility to environmental and lifestyle-related reproductive hazards. It might be difficult to distinguish the subtle effects of smoking from other

factors in subjects with seriously impaired semen quality (Vogt *et al.*, 1986; Saaranen *et al.*, 1987).

Recent studies have also shown that maternal smoking during pregnancy might have an impact on the reproductive organs of the progeny, resulting in decreased sperm production and increased risk for testicular cancer (Akre *et al.*, 1996; Ekbom & Akre, 1998; Kaijser *et al.*, 2003; Storgaard *et al.*, 2003; Jensen *et al.*, 2004b, 2005; Pettersson *et al.*, 2004). Furthermore, in Sweden, almost 19% of young males snuff tobacco (Holm *et al.*, 1992; Fagerström & Schildt, 2003). However, the effects of snuffing on male reproductive function are unknown.

In this study, we mainly focused on the effect of current smoking on the reproductive function of young healthy males from the general population. Additionally, we wished to investigate whether snuffing and maternal smoking during pregnancy might have an impact on male reproductive parameters, either directly or as confounders or as effect modifiers.

Subjects and methods

Subjects, samplings and clinical examination

During May to December 2000 all 2255 men presenting for the compulsory medical board prior to military service and living within 60 km from Malmö in southern Sweden were asked to participate in a study on reproductive function in young Swedish males (Richthoff *et al.*, 2002a). Of the 305 (13.5%) who agreed to participate, three were excluded; one for not giving information about his smoking habit and two because of cannabis smoking. Thus 302 men participated in the study; among them two were unable to deliver a semen sample and in one subject blood sampling for hormone analysis failed. Thus, the study group included 300 subjects for the semen study and 301 for the hormone analyses. The median age of the participants was 18 years (range 18–21).

For hormone analyses, blood samples were drawn between 8:00 and 10:00 h from a cubital vein. The serum was frozen at −80 °C and analysed within 6 months. All subjects underwent an andrological examination, where one doctor (J.R.) examined 90% of all the men and another examiner (A.G.) saw the remaining 10%. Both were experienced in andrological examination. The examination included a genital investigation. The testicular size was measured by use of ultrasound, and immediately thereafter the men delivered a semen sample. All participants filled in a questionnaire at home regarding their smoking (smoking: yes/no; if 'yes': how many cigarettes per day), drinking habits and possible incidence of congenital abnormalities (Table 1). The questionnaire also contained questions on their mothers' tobacco smoking during pregnancy (Table 1). Of the whole cohort of conscripts ($n = 302$), 217 were non-smokers, whereas the remaining 85 were smokers. Data on the mothers' smoking habits (yes/no) were available from 269 subjects. Fifty-four reported that their mother smoked during pregnancy whereas 215 were non-smokers. In Sweden, snuffing is another common way of tobacco exposure. As the original questionnaire did not have questions regarding snuffing, we contacted the participants by telephone after completing data collection. Among the 242 who were reached, 51 stated that they were snuffers at the time of sample delivery.

Table 1 Background characteristics for the 302 military conscripts

	Non-smokers ($n = 217$)	Smokers ($n = 85$)	Total ($n = 302$)
Mean (SD)			
Age (years)	18.1 (0.4)	18.2 (0.4)	18.1 (0.4)
Abstinence time (h)[a]	89 (63)	76 (37)	85 (57)
BMI (kg/m^2)[a]	22.6 (3.0)	22.4 (3.5)	22.6 (3.2)
Alcohol consumption (g/week)	42 (123)	36 (47)	41 (107)
N (%)			
Diseases in reproductive organs[b]	48 (21)	15 (18)	63 (21)
Mother smoking during pregnancy[c] (yes)	37 (17)	17 (20)	54 (18)
Snuffing[d]	32 (15)	19 (22)	51 (17)
Season			
Summer	45 (21)	19 (22)	64 (21)
Autumn	110 (51)	43 (51)	153 (51)
Winter	18 (8)	6 (7)	24 (8)
Spring	44 (20)	17 (20)	61 (20)

[a]Information from 299 conscripts.
[b]Including varicocele, hydrocele, retentio testis, testis torsion, infections and inguinal hernia.
[c]Data regarding 269 mothers.
[d]Data from 242 conscripts.

All men participated after given written informed consent according to protocols approved by the ethical review board of Lund University.

Semen analysis

All subjects were asked to be abstinent for at least 48 h. In each case the actual length of the abstinence period was registered (Table 1). Semen volume and sperm concentration as well as motility were analysed according to World Health Organization (1999) recommendations. Three laboratory technicians preformed analyses of the ejaculates, and the laboratory participated in the Nordic Association of Andrology and ESHRE (European Society of Human Reproduction and Embryology) quality control programme (Cooper *et al.*, 2002). The inter-observer

coefficient of variation (CV) was found to be 8.5% for concentration assessment. The percentages of semen samples delivered during different seasons were: 21.1% summer (June 21–September 20); 51.3% autumn (September 21–December 20); 7.3% winter (December 21–March 20); and 20.3% spring (March 21–June 20).

Hormone analyses

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), sexual hormone binding globulin (SHBG), testosterone and oestradiol were measured on an automated fluorescence detection system (Autodelfia; Wallac Oy, Turku, Finland). Intra- and total-assay CV were below 4% and 7.5% respectively. Inhibin B levels were assessed using a specific immunometric assay as described previously (Groome *et al.*, 1996) with a detection limit of 15 ng/L and total-assay CV below 7%.

Biochemical seminal markers

We analysed neutral-alpha glycosidase (NAG) marker for epididymal function, fructose marker for seminal vesicle function and the markers for prostate function, prostate-specific antigen (PSA) and zinc. The concentrations was measured in the seminal plasma as reported previously (Richthoff *et al.*, 2002b). The NAG concentration was measured using a commercially available kit (Episcreen®; Fertipro, Berne, Belgium), with a CV of 15%, while the PSA concentration was determined with a fluoroimmunometric method (PROSTATUS™ kit; Wallay Oy), with a CV of 5%. The concentration of fructose was determined with a spectophotographic method, described by Wetterauer & Heite (1976), run on a Beckman (Brea, California) Synchron LX20 instrument. The CV was 5% for control samples. Zinc concentration was assessed with a colorimetric method (Makino *et al.*, 1982), with a CV of 7%.

Statistical analysis

Linear regression analysis was used when the different reproductive parameters were compared between smokers and non-smokers, and between those who smoked 1–9 cigarettes/day and those who smoked ≥10 cigarettes/day respectively. If the *p*-values were ≤0.1, linear regression analysis was also performed with number of cigarettes as a continuous variable. We also evaluated the effect of snuffing (yes/no) and maternal smoking (smokers/non-smokers) with respect to the reproductive parameters, using linear regression analysis. If visual examination of the residual plots indicated skewness, logarithmic transformation of data was tested. Eventually, logarithmic transformations were applied for sperm concentration, total sperm count, FSH and the biochemical biomarkers markers NAG, fructose, PSA and zinc. For each of the three routes of tobacco exposure (i.e. current smoking, current snuffing and maternal smoking), the two others were considered as potential confounders. Furthermore, alcohol intake (three categories: 0, 1–49 and ≥50 g/week) and the length of abstinence period (categories: <48, 49–72, 73–96 and ≥97 h) were also considered as potential confounders. If the adjusted estimates differed by less than 15% from the crude estimate, we only presented the crude results. We also tested whether current smoking, snuffing and maternal smoking modified each others' effect by including pair-wise interaction terms in the models. Genital abnormalities – self-reported or found at the time of examination – did not differ in frequency between smokers and non-smokers and were not taken into consideration (Table 1). The same was true as regards the effect of season (Table 1), the percentage of smokers being constant throughout the four seasons.

Results

Semen variables and testicular volume

There were no differences between non-smokers and smokers regarding testicular volume ($p > 0.5$, Table 2), sperm concentration ($p = 0.15$) or motility ($p > 0.5$). However, when comparing the total sperm count, the mean value was 41% (95% CI 4.8–88%, $p = 0.02$) higher among non-smokers than smokers. The same trend, although not statistically significant, was noted with respect to seminal volume, with 0.3 mL (95% CI −0.03 to 0.6 mL, $p = 0.08$) higher volume among non-smokers. No statistical differences for any of these outcome variables were found between the subjects smoking 1–9 cigarettes and subjects smoking >9 cigarettes a day.

When including snuffing and maternal smoking in the models, the difference in sperm concentration between smokers and non-smokers became more pronounced (mean difference: 37%, 95% CI −4% to 95%, $p = 0.08$), although not statistically significant. For the total sperm count the difference between the two groups was more pronounced (49%; 95% CI 4.5–112%, $p = 0.01$).

Neither maternal smoking (Table 3) nor snuffing seemed to have any independent impact on the semen parameters and no statistically significant interactions between the three types of tobacco exposure were seen.

Seminal biochemical biomarkers

Compared with smokers, non-smokers had higher amounts of the prostate markers PSA (19%, 95% CI 2.9–39%, $p = 0.02$) and zinc (29%, 95% CI 5.9–57%, $p = 0.01$) per ejaculate (Table 4). There was also a trend

Table 2 Testicular volume and semen characteristics (crude values) in 302 military conscripts, grouped according to their smoking habits

		Smokers			*p*-Value	
	Non-smokers ($n = 217$)	All ($n = 85$)	1–9/day ($n = 47$)	≥10/day ($n = 38$)	Non-smokers vs. smokers	≤9 cig. vs. ≥10 cig./day
Testicular volume (mL)	29 (7.4)	29 (8.3)	28 (7.3)	31 (9.2)	>0.5	0.13
Seminal volume (mL)[a]	3.3 (1.3)	3.0 (1.4)	3.0 (1.2)	2.9 (1.5)	0.08	0.45
Sperm concentration ($\times 10^6$/mL)[b]	75 (67)	66 (65)	64 (51)	67 (79)	0.15	>0.5
Total sperm count ($\times 10^6$)[c]	224 (198)	163 (126)	168 (116)	157 (139)	0.02	>0.5
Total progressive motile[c]	48 (23)	50 (22)	49 (22)	51 (21)	>0.5	>0.5
CASA (% motile)[b]	46 (25)	48 (24)	49 (24)	47 (23)	>0.5	>0.5

Values are presented as mean (SD). CASA, computer-aided sperm analysis.
[a]Only information from 300 conscripts.
[b]Only information from 282 conscripts.
[c]Only information from 298 conscripts.

Table 3 Testicular volume and semen characteristics (crude values) in 269 military conscripts, grouped according to their mother's smoking habits during pregnancy

		Non-smoking mother ($n = 215$)	Smoking mother ($n = 54$)	*p*-Value (non-smoking mother during pregnancy vs. smoking)
	Available data from	Mean (SD)		
Testicular volume (mL)	269	29 (7.9)	30 (8.1)	0.29
Seminal volume (mL)	269	3.3 (1.3)	3.0 (1.4)	0.1
Sperm concentration ($\times 10^6$/mL)	267	71 (63)	74 (74)	>0.5
Total sperm count ($\times 10^6$)	267	216 (185)	201 (194)	>0.5
Total progressive motile (%)	267	47 (23)	49 (22)	>0.5
CASA (% motile)	252	46 (25)	47 (26)	>0.5

CASA, computer-aided sperm analysis.

Table 4 Total amount of seminal markers (crude value) per ejaculate in smoking and non-smoking Swedish military conscripts ($n = 288$)

		Smokers			*p*-Value[a]	
	Non-smokers ($n = 206$)	All ($n = 82$)	1–9/day ($n = 46$)	≥10/day ($n = 36$)	Non-smokers vs. Smokers	≤9 cig. vs. ≥10 cig./day
Total amount per ejaculate	Mean (SD)					
NAG (mU)[b]	22.6 (1.8)	19.3 (1.9)	18.8 (1.8)	20.1 (2.0)	0.07	>0.5
Fructose (μmol)	42.3 (2.0)	36.0 (2.3)	36.4 (2.4)	35.3 (2.3)	0.10	>0.5
PSA (mg)	1973 (1.8)	1653 (1.8)	1654 (1.9)	1651 (1.8)	0.02	>0.5
Zinc (μmol)	4.3 (2.1)	3.4 (2.1)	3.1 (2.3)	3.7 (1.9)	0.01	0.36

NAG, neutral-alpha glycosidase; PSA, prostate-specific antigen.
[a]Adjusted for potential confounders using multiple regression models.
[b]Only available from 251 conscripts.

towards a higher amount of the epididymal marker NAG (17%, 95% CI: −2% to 39%, $p = 0.07$) and the seminal vesicle marker fructose (18%, 95% CI −4% to 43%, $p = 0.1$) in the non-smoker group. There were no differences for these biomarkers between the subgroups smoking 1–9 cigarettes and >9 cigarettes/day.

After adjusting for maternal smoking, the significant association between non-smokers and smokers in PSA did not persist (12%, 95% CI −4% to 32%, $p = 0.15$), and the association with respect to zinc became weaker (23%, 95% CI −1% to 51%, $p = 0.06$).

Men with a non-smoking mother during pregnancy had 26% higher (95% CI 2.8–56%, $p = 0.03$) total amount of NAG and 48% higher (95% CI 18–86%, $p = 0.001$) total amount of fructose per ejaculate than men with smoking mothers. There were no statistically

significant associations between snuffing and seminal biochemical biomarkers. No statistically significant interaction between the tobacco variables was noted for any of the outcome variables.

Hormones

The non-smokers had on average 17% higher (95% CI 3.1–33%, $p = 0.02$) concentration of FSH than smokers (Table 5). This association was due to the pronounced low concentrations of FSH among men who smoked >9 cigarettes a day. FSH concentration in this subgroup was 37% lower (95% CI 10–69%, $p = 0.005$) when compared with men who smoked 1–9 cigarettes/day. The concentration of inhibin B did not differ between non-smokers and the whole group of smokers. However, the subgroup of men who smoked >9 cigarettes had a nonsignificant tendency of higher inhibin B concentration when compared with those smoking 1–9 cigarettes/day (mean difference 24 ng/mL, 95% CI −51 ng/mL to 1.9 ng/mL, $p = 0.07$). With respect to other hormones, no significant associations were found with smoking status.

When adjusting for snuffing, the association in FSH between those who smoked 1–9 cigarettes/day and those who smoked >9 cigarettes/day became somewhat more pronounced (57%, 95% CI 21–100%, $p = 0.001$).

When using cigarettes per day as a continuous variable, the associations with both FSH and inhibin B were still statistically significant. When we included snuffing in the models, an increase of one cigarette per day corresponded to a 3.1% lower (95% CI 1.6–4.3%, $p = 0.0001$) concentration of FSH and 1.8 ng/mL higher inhibin B values (95% CI 0.10–3.6 ng/mL, $p = 0.04$).

Using snuffing and maternal smoking separately as hypothesis variables did not show any statistically significant associations with hormone levels, irrespective of confounder adjustment, and neither was there any significant interaction between the three tobacco variables. The adjusted R^2 values for statistically significant associations throughout the study varied from 0.01 to 0.14.

Discussion

In the current study we found significantly reduced total sperm counts in smokers when compared with non-smokers, while smoking status did not affect sperm density or motility. Moreover, smoking was associated with a dose-dependent decrease in serum FSH. Furthermore, those who had a mother who smoked during pregnancy had lower seminal excretion of NAG and fructose. Snuffing was not associated with any of the reproductive parameters. However, generally, tobacco exposure could only explain a minor part of the variation in reproductive parameters, the adjusted R^2 values being between 0.01 and 0.14.

This study was based on a large group of healthy males with a median age of 18 years, which can be regarded as representative of Swedish male adolescents. A 27% prevalence of smokers is in accordance with the fraction of smokers recorded in recent cohorts of conscripts in southern Sweden (http://www.pliktverket.se). The percentage of smokers in Danish conscripts at this time was 43% (N. Jorgensen, personal communication). Different smoking habits among young Swedish and Danish conscripts could be one of the factors contributing to the differences in sperm parameters found in a previous study (Richthoff *et al.*, 2002a). In this study Swedish conscripts had on average 50 millions more sperms in the ejaculate compared with their Danish counterparts. Based on our findings of an average of 73 millions more sperms per ejaculate in non-smokers when compared with smokers, a 16% discrepancy in the fraction of smokers might account for no more than 20% of the difference in sperm number between the Swedish and the Danish conscripts.

In Sweden, 95% of all males undergo medical health examination prior to military service. Only those with serious and chronic diseases are excluded. Despite the participation rate of only 13.5%, we do not believe that we have a significant selection bias in our study, at least as regards the reproductive function of the participants. The reason for this is that most men in this age are

Table 5 Comparison of reproductive hormone levels (crude values) in smoking and non-smoking Swedish military conscripts ($n = 301$)

		Smokers			*p*-Value	
	Non-smokers ($n = 216$)	All ($n = 85$)	1–9/day ($n = 47$)	≥10/day ($n = 38$)	Non-smokers vs. smokers	≤9 cig. vs. ≥10 cig./day
Testosterone (nmol/L)	23 (5.3)	23 (5.4)	23 (5.4)	24 (5.4)	0.38	0.51
SHBG (nmol/L)	28 (9.6)	29 (9.8)	27 (8.5)	31 (11.1)	>0.5	0.15
FSH (IU/mL)	3.1 (1.6)	2.7 (1.7)	3.1 (1.6)	2.2 (1.7)	0.02	0.005
LH (IU/mL)	4.2 (1.5)	4.5 (1.7)	4.4 (1.7)	4.5 (1.8)	0.25	>0.5
Inhibin B (ng/mL)	208 (61)	214 (62)	203 (56)	227 (66)	0.42	0.07

Values are presented as mean (SD). SHBG, sexual hormone binding globulin; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

usually not aware of their reproductive capability. Furthermore, in a study of Danish military conscripts by Andersen *et al.* (2000), having a participation rate similar to ours, levels of reproductive hormones in the circulation of those who provided a semen sample were similar to those not willing to deliver a sample. It was, therefore, suggested that these two groups did not differ from a reproductive point of view. Because of their youth, it is plausible to believe that the men in our study had experienced a relatively short history of tobacco use. Nevertheless, it seemed to have had an impact on their sperm production which cannot be neglected. Taking into account the effect of possible confounders as time of abstinence, maternal smoking habits, alcohol intake and snuffing, the effect of smoking on sperm numbers remained unchanged. Seasonal differences in semen parameters have been suggested (Chen *et al.*, 2003; Chen *et al.*, 2004) with highest sperm concentrations in winter and autumn. However, other studies could not confirm such findings (Malm *et al.*, 2004). In the present study we did not treat seasonal variation as a potential confounder as in a previous study, based on the same study population, we did not find any association between season and male reproductive parameters (Richthoff *et al.*, 2002a). Similarly, there was no difference between smokers and non-smokers as regards the frequency of genital abnormalities – self-reported or found at the time of examination. We do not have any information on caffeine intake. Previous studies found no strong impact of caffeine on semen quality (Pichini *et al.*, 1994; Loft *et al.*, 2003).

Some recent studies (Storgaard *et al.*, 2003; Jensen *et al.*, 2004b; Jensen *et al.*, 2005) have reported reduced sperm counts among men whose mothers smoked during pregnancy. In our study maternal smoking during pregnancy did not have any effect on the sperm numbers of the progeny. We relied on retrospective information from the sons, while a Danish study (Storgaard *et al.*, 2003) had access to data on maternal smoking from a medical birth register. The data published by Jensen *et al.* (2004a,b) were also based on military conscripts. However, the Danish study included almost 1800 subjects, giving it a higher statistical power when compared with the current study.

As a novel finding, lower amount of seminal NAG and fructose was seen among the sons of smoking mothers. This finding might indicate an effect of maternal smoking during pregnancy on epididymal and seminal vesicle function in the progeny. The function of accessory sex glands is reflected through the motility percentage of the spermatozoa (ELzanaty *et al.*, 2002) and reduced sperm motility found in the sons of smoking mothers (Jensen *et al.*, 2004b) might be due to the negative effects of smoking on the function of post-testicular organs (i.e. epididyemis, seminal vesicle and prostate).

The negative impact of smoking was most pronounced with respect to total sperm count whereas the effect on sperm concentration was less pronounced and not statistically significant. Thus, unchanged sperm concentration despite decreased total sperm count might be the result of a trend towards lower seminal volume in smokers. Accordingly, in a recently published study (Pasqualotto *et al.*, 2006), lower seminal volume was found among vasectomy candidates smoking more than 20 cigarettes/day. The lower seminal volume might be due to decreased prostate function in smokers, a phenomenon previously reported (Pakrashi & Chatterjee, 1995), but not confirmed in the present study.

Despite the high prevalence of cigarette smoking in the general population, our knowledge of its impact on male reproductive function is still very limited. In a meta-analysis, smoking was only found to have an adverse effect on sperm concentration and motility in healthy volunteers and sperm donors, but not in infertility patients (Vine *et al.*, 1994).

The mechanism behind the reduction in sperm production among smokers is not yet understood (Russel *et al.*, 1981). We did not find any effect of snuffing on any of the reproductive parameters evaluated. This might indicate that it is not the tobacco itself which causes the negative impact on reproductive parameters but rather the compounds which are released by smoking.

Polycyclic aromatic hydrocarbons – one of the compounds of cigarette smoke but not released by snuffing – has been found to reduce fertility in both male and female mice (Mackenzie & Angevine, 1981) and to have a negative impact on Sertoli cell function (Raychoudhury & Kubinski, 2003). The gonadotoxic effect of smoking might also be mediated through oxidative damage (Shen *et al.*, 1997), which is potentially hazardous for spermatogenesis.

Polycyclic aromatic hydrocarbons were also shown to impair Leydig cell production of testosterone (Inyang *et al.*, 2003). The finding of an inverse correlation between smoking and prostate size (Kupeli *et al.*, 1997) could also point to an anti-androgenic effect of cigarette smoking. It was recently shown that late stages of spermatogenesis are testosterone-dependent (Raychoudhury & Kubinski, 2003; De Gendt *et al.*, 2004), and sperm production might, therefore, be impaired due to lower intratesticular testosterone levels. As the concentration of this hormone within the testis is approximately 100 times higher than in plasma, slight impairment of Leydig cell function might affect spermatogenesis without any changes in peripheral hormone levels.

Usually, a decrease in sperm counts is associated with rising FSH concentration and lower levels of inhibin B. In our study we found significantly higher FSH levels in

non-smokers when compared with smokers, whereas concentrations of LH, inhibin B, testosterone and SHBG did not differ between the two groups. There was no statistical difference in FSH levels among non-smokers and those smoking 1–9 cigarettes per day. However, comparing non-smokers and those smoking 10 cigarettes or more we found statistically significantly higher FSH levels among the non-smokers. When we compared inhibin B levels no difference was obtained between non-smokers and smokers but the number of cigarettes correlated positively with the concentration of this hormone. Interestingly, Jensen *et al.* (2005) reported a trend towards positive association between maternal smoking during pregnancy and serum inhibin B levels in the male offspring, despite an inverse correlation between cigarette consumption of the mothers and sperm number in the male offspring. Although the mechanism behind the smoking-related increase in inhibin B concentration and the reduction in sperm production remains unresolved, one could speculate whether cigarette exposure might imply an impairment of the post-meiotic events in spermatogenesis and consequently, due to some yet unknown paracrine effects, evokes an increase in the levels of Sertoli cell-derived inhibin B and subsequent reduction in FSH concentration. Previously, we reported high inhibin B levels in subjects with post-meiotic impairment of spermatogenesis due to mild androgen insensitivity (Giwercman *et al.*, 2000). Other studies have indicated increased FSH levels in male smokers over 30 years of age. Therefore, it cannot be excluded that long-term smoking may lead to more profound impairment of sperm production, also affecting the early stages of sperm production.

We found a negative effect of maternal smoking on epididymal and seminal vesicle function, which has not been reported previously. If this is a true biological phenomenon, and not a serendipitous finding, the effect of maternal smoking might be directly toxic on these organs. The effect of smoking on post-testicular organs might also be mediated through impairment of Leydig cell function (Yamamoto *et al.*, 1998) as mentioned above.

In conclusion, we found a negative effect of current smoking and maternal smoking during pregnancy on the reproductive parameters of Swedish adolescent men. The results of this study should be seen in view of our recent report on significantly higher sperm counts in Sweden compared with Denmark (Richthoff *et al.*, 2002a), as cigarette smoking is much more common among Danish males than Swedish males (Manninen, 1997). Hence, cigarette smoking might, at least partly, contribute to the reported geographical differences in sperm numbers.

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