

# Is smoking a risk factor for decreased semen quality? A cross-sectional analysis

C.H.Ramlau-Hansen<sup>1</sup>, A.M.Thulstrup, A.S.Aggerholm, M.S.Jensen, G.Toft and J.P.Bonde

Department of Occupational Medicine, Aarhus University Hospital, Aarhus Sygehus, Denmark

<sup>1</sup>To whom correspondence should be addressed at: Department of Occupational Medicine, Aarhus University Hospital, Aarhus Sygehus, Norrebrogade 44, building 2C, DK-8000 Aarhus C, Denmark. E-mail: craha@as.aaa.dk

**BACKGROUND:** Previous studies suggest a deleterious effect of cigarette smoking on semen quality, but their results have not been consistent. We studied the association between current smoking and semen characteristics and hormonal levels in a large group of healthy men. **METHODS:** From 1987 to 2004, seven separate occupational or environmental semen quality studies were co-ordinated by our department. A total of 2562 men participated, each providing semen and blood sample and answering a questionnaire about lifestyle and factors related to health. Appropriate semen and smoking data were available for 2542 men. **RESULTS:** Adjusting for study, age and other covariates, we observed an inverse dose–response relation between smoking and semen volume, total sperm count and percentage motile sperm. Heavy smokers had a 19% lower sperm concentration than non-smokers. We found a positive dose–response relationship between smoking and testosterone, LH and the LH/free testosterone ratios. **CONCLUSION:** Current smoking in adult life moderately impairs the semen quality. It is well known that semen quality is associated to fecundity. Therefore, it would be sensible to advise men to abstain from smoking to avoid decreased fecundity.

**Key words:** current smoking/prenatal exposure/reproductive hormones/sperm concentration/total sperm count

## Introduction

The effect of smoking on semen quality has been investigated in a number of cross-sectional studies, most of which have included infertility patients. Their results are conflicting: some report smokers to have a lower semen quality in terms of the conventional semen characteristics (semen volume, sperm concentration, total count, motility and morphology), whereas others report no effect of smoking. A meta-analysis by Vine *et al.* (1994) showed that smokers' sperm concentration on average was 13% lower than that of non-smokers. Among normal healthy men (i.e. excluding infertility clinic patients), smokers had ~24% lower sperm concentration than non-smokers (Vine *et al.*, 1994). A mini-review by Marinelli *et al.* (2004) concluded that smoking has limited effect on semen quality. Two recent investigations reported inconsistent results: smokers had ~15% lower sperm concentration and 18% lower total sperm count than non-smokers in a large cross-sectional study of infertile couples (Kunzle *et al.*, 2003), whereas current smoking had no independent effect on semen quality in a large sample of young men from five different European countries (Jensen *et al.*, 2004).

A possible dose–response relationship between increased number of cigarettes smoked per day and decreased semen quality has been investigated in 18 studies. Their results were, however, mostly statistically non-significant and inconsistent.

Six studies were performed in healthy men (Spira *et al.*, 1981; Vogt *et al.*, 1986; Saaranen *et al.*, 1987; Vine *et al.*, 1994, 1996; Pasqualotto *et al.*, 2006), and 12 studies were conducted in infertility clinic patients (Evans *et al.*, 1981; Rodriguez-Rigau *et al.*, 1982; Andersen *et al.*, 1984; Rantala and Koskimies, 1987; Marshburn *et al.*, 1989; Oldereid *et al.*, 1989, 1992; Lewin *et al.*, 1991; Osser *et al.*, 1992; Merino *et al.*, 1998; Al Bader *et al.*, 1999; Zhang *et al.*, 2000). They all suggested a decline in semen volume, sperm concentration, motility or morphology with an increase in the number of cigarettes smoked per day.

The relationship between cigarette-years (i.e. number of cigarettes per day multiplied by the number of years of smoking) and semen quality has been assessed in two studies by Chia *et al.* (1994a,b) and one study by Wang *et al.* (2001). The two studies by Chia *et al.* reported an inverse dose–response relationship between cigarette-years and sperm concentration. The study by Wang *et al.*, however, reported an inverse association for years smoked and sperm concentration but not for cigarette-years and sperm concentration. Finally, the relationship between cotinine (the major nicotine metabolite) in seminal plasma and semen characteristics has been evaluated in three studies, which reported an inverse correlation between cotinine and sperm concentration, motility, morphology or total sperm count (Pacifici *et al.*, 1993; Sofikitis *et al.*, 2000; Wong *et al.*, 2000).

Most previous studies of the relationship between smoking and semen quality have been performed in infertile and relatively small groups, and large-scale studies reflecting the variety of smoking patterns among healthy men are therefore needed. The aim of this study is accordingly to investigate the dose–response relationship between current cigarette smoking and conventional semen characteristics and hormonal levels in a large group of healthy men.

## Materials and methods

### Populations

From 1987 to 2004, seven separate semen quality studies were co-ordinated by the Department of Occupational Medicine, Aarhus University Hospital, Aarhus, Denmark. A total of 2562 men participated (Table I). Five studies were occupational semen quality studies of which four comprised Danish men (the Metalworker, Planner, Gardener and Farmer studies) (Bonde, 1990; Bonde *et al.*, 1998b; Larsen *et al.*, 1999; Abell *et al.*, 2000), and one comprised men from the UK, Italy and Belgium (the Lead worker study) (Bonde *et al.*, 2002). Another study investigated the effect of prenatal exposure to estrogen on semen quality in Danish men (the Twin study) (Storgaard *et al.*, 2002), and one study investigated the effect of exposure to organochlorines on semen quality in men from Greenland, Poland, Sweden and Ukraine (the Inuendo study) (Toft *et al.*, 2005), which is a pregnancy cohort from the general population.

The men ranged in age from 17 to 67 years and met the following inclusion criteria: no vasectomy, no known azoospermia and no abnormalities in the reproductive organs. In the Planner study, couples were eligible for the study if they had no reproductive experience and intended to stop contraception and have children (Bonde *et al.*, 1998b).

We were able to identify appropriate semen (sperm concentration) and smoking data for 2542 men (99.2%), who formed the study population in the present study. Ethical approval was obtained by local ethical committees. Study design, selection of participants and detailed description of the seven original studies have previously been described in detail (Bonde, 1990; Bonde *et al.*, 1998b, 2002; Larsen *et al.*, 1999; Abell *et al.*, 2000; Storgaard *et al.*, 2002; Toft *et al.*, 2005).

### Exposure and outcome data

Information about current smoking habits was obtained with comprehensive self-completed questionnaires. The questions about smoking habits were basically the same among the seven studies. The participants noted exactly how many cigarettes, cheroots, cigars and grams of pipe tobacco they smoked per day. Answering categories were not

used. Tobacco sources other than cigarettes were converted into 'number of cigarettes smoked per day' according to estimated tobacco content: cigarette, 0.9 g of tobacco; cheroot, 4.1 g of tobacco and cigar, 7.3 g of tobacco (Skandinavisk Tobakskompagni, personal communication). Information on reproductive, medical, occupational and lifestyle factors was also obtained by questionnaire.

Blood samples were collected by venipuncture when the participants delivered the semen samples. The samples were stored at  $-20$  or  $-80^{\circ}\text{C}$  until analysis. Reproductive hormones were measured by standard immunometric techniques as described in the respective articles.

The participants produced semen samples by masturbating into 50-ml polyethylene jars. The samples were collected at the participants' homes and kept close to the body during transportation to avoid cooling. The samples were analysed either in a mobile laboratory or in a hospital, and the examination of 63% of the samples was initiated within the first hour, where it has been shown that the motility is stable (Makler *et al.*, 1979).

Semen volume was measured in a graded tube with 0.1-ml accuracy or in a balance. Sperm motility was assessed after liquefaction by grading the sperm cells as either motile (grade a and b) or immotile (grade c and d). Sperm concentration was counted in a Makler, a Neubauer or a Bürger-Türk chamber using a phase-contrast microscope. The use of different counting chambers has by some been shown to produce similar sperm concentration results (Auger *et al.*, 2000), whereas others have found marked differences between the chambers (Mahmoud *et al.*, 1997). For this reason, we initially performed stratified analysis according to chamber (Neubauer versus Makler and Bürger-Türk), and chamber did not modify the association between smoking and sperm concentration. Trained medical laboratory technicians performed all the analyses in accordance with the successive editions of the guidelines published by the World Health Organization (1980–1999) in its 'Laboratory manual for the examination of human semen and sperm–cervical mucus interaction' (World Health Organization, 1999).

Information about the duration of sexual abstinence, spillage during collection and fever within the last 3 months was entered in a questionnaire filled in by the participants themselves when collecting the semen samples. The number of semen samples from each participant differed among the seven studies, but in this study, we only used the first sample from each.

### Statistical analysis

Current smoking (the explanatory variable) was divided into four strata equivalent to no smoking (59%), 1–10 cigarettes/day (light smoking) (17%), 11–20 cigarettes/day (medium smoking) (20%) and >20 cigarettes/day (heavily smoking) (4%).

Outcome variables included semen volume (ml), sperm concentration ( $\times 10^6/\text{ml}$ ), total sperm count (concentration  $\times$  volume,  $\times 10^6$ ),

**Table I.** Year of study, participation rates and number of participants in the seven occupational and environmental studies

Study subgroups	Number of participants <sup>a</sup>	Participation rate <sup>b</sup> (%)	Year of study	Reference
Metalworkers	136	37	1986–1987	Bonde (1990)
Planners	415	16	1992–1995	Bonde <i>et al.</i> (1998b)
Gardeners	152	61	1994	Abell <i>et al.</i> (2000)
Farmers	256	32	1995–1996	Larsen <i>et al.</i> (1999)
Lead workers	485	18	1996–1997	Bonde <i>et al.</i> (2002)
Twins	302	39	1999–2000	Storgaard <i>et al.</i> (2002)
Inuendo	796	18	2002–2004	Toft <i>et al.</i> (2005)
Total	2542			

<sup>a</sup>Number of participants with information on current smoking and sperm concentration from original studies included in this study. In total, 20 participants from the original studies were excluded due to missing information: 3 from the Planner study, 1 from the Lead study, 14 from the Twin study and 2 from the Inuendo study.

<sup>b</sup>Participation rate in original studies. The participation rate in the Planner study is an estimate.

percentage of motile sperm cells (motility grade a and b), serum levels of testosterone (nmol/l), estrogen (pmol/l), inhibin B (pg/ml), FSH (IU/l), LH (IU/l) and sex hormone-binding globulin (SHBG, nmol/l).

Crude median, 25th (p25) and 75th (p75) percentiles were calculated for all outcome variables. The non-parametric Spearman's rank correlation test was used to test for trend between the increased smoking stratum and a decrease (or increase) in the outcome variables. The Kruskal–Wallis test was applied to test for overall association between the four smoking strata and the outcome variables.

Because the data on percentage of motile sperm were normally distributed, crude means and 95% confidence intervals (CIs) are presented. Data on semen volume, sperm concentration, total sperm count, testosterone, estrogen, SHBG and inhibin B were cubic-root transformed to normalize the distribution, and crude back-transformed means with CIs were calculated. Data on FSH and LH were transformed by the natural logarithm to normalize the distribution, and crude geometric means with CIs were calculated.

For each of the outcome variables, we performed a generalized multiple linear regression analysis, using the four strata of current smoking as a categorical explanatory variable. When testing for trend, the four smoking strata were treated as a continuous explanatory variable. We controlled for relevant confounders as described below. The adjusted results are presented as back-transformed means with 95% CIs. The following characteristics were chosen as reference in the models: the Planner study, age 20 years, 4 days of abstinence, no fever within the last 3 months, sampling between October and March, no diseases in reproductive organs, normal weight (BMI between 20 and 25 kg/m<sup>2</sup>), no daily coffee consumption, no prenatal exposure to tobacco smoke and blood sampling between 0600 and 1200 h. Participants who reported spillage when sampling were excluded from all statistics on semen volume and total sperm count.

Possible confounding variables were identified and grouped into obligate and potential covariates. Obligate covariates that were kept in all the semen models were study group (categorical), age (continuous, ln-transformed) and abstinence time (continuous, ln-transformed). Additionally, in the sperm concentration model, spillage when collecting the sample (yes/no/do not know) was an obligate covariate and so was time from ejaculation to analysis (continuous, ln-transformed) in the motility model. In the blood sample models, study group, age and time of day for sampling (0900–1200/1201–0859 h) were entered as obligate covariates. Obligate covariates were included in the model regardless of their effect. Other potential confounders included fever (>38°C) within 3 months of sampling (yes/no/do not know), season of sampling (April–September/October–March), diseases in the reproductive organs (varicocele, hydrocele, testicular cancer, orchitis and cryptorchism combined into one variable, present or not present), BMI (<20, 20–25, >25 kg/m<sup>2</sup>), daily coffee consumption (yes/no) and prenatal tobacco smoke exposure (yes/no/do not know). The potential confounders were entered forward stepwise in the model, and if they did not change the estimate (the slope,  $\beta$ ) by at least 10%, they were removed from the final model. Because of variety in the original studies, all variables were not recorded, and much information was therefore missing, for example on BMI (569 missing), daily coffee consumption (1541 missing) and prenatal tobacco smoke exposure (959 missing). We decided not to include information on BMI, coffee consumption and prenatal tobacco smoke exposure in the main multiple regression analysis, as it would have considerably reduced the power of the study. Sub-analysis including information on BMI, coffee consumption and prenatal tobacco smoke was performed.

We evaluated the fit of the regression models by inspecting the residual and leverage plots.

Each final model was checked for study subgroup interaction, and study subgroup had no statistically significant modifying effect of current smoking in any of the models.

Finally, the sperm concentration was dichotomized in two ways: as oligozoospermia (>0 and <20 × 10<sup>6</sup>/ml) and normospermia (≥20 × 10<sup>6</sup>/ml) and as azoospermia (0 × 10<sup>6</sup>/ml) and normospermia. Logistic regression analysis was performed, and crude and adjusted odds ratios (ORs) for oligozoospermia and azoospermia in relation to current smoking were calculated. Azoospermic men were excluded from the oligozoospermia analysis, and oligozoospermic men were excluded from the azoospermia analysis.

All statistics were performed by using the 'Intercooled Stata 8.2' software package. A two-tailed probability level of <0.05 was chosen as the level of statistical significance.

## Results

Characteristics of the 2542 participants stratified according to current smoking are summarized in Table II. Compared with the non-smokers, smokers were older, more often coffee drinkers, more often harboured diseases of the reproductive organs and had more often been exposed to maternal smoking during pregnancy. They also often had their blood collected outside the time interval 0600–1200 h and had their semen collected in the period between April and September than non-smokers.

### *Semen quality and hormonal levels in relation to current smoking*

The crude median sperm concentration fell with increased smoking from 57.5 × 10<sup>6</sup>/ml (p25–75: 28–98) among the non-smokers to 41.0 × 10<sup>6</sup>/ml (p25–75: 22–82) among the heavily smoking men, which is equivalent to a 29% decrease (Table III). After transformation and after controlling for age, study and other covariates as described in the footnotes to Table III, the inverse dose–response relationship between the number of cigarettes smoked per day and the mean sperm concentration was less pronounced, although the heavily smoking men still had a 19% lower mean sperm concentration than the non-smokers.

There was a trend towards decreased adjusted mean semen volume, total sperm count and sperm motility with increased smoking. Heavily smoking men had a 29% lower mean total sperm count and a 13% lower mean percentage motile sperm than non-smoking men.

A positive trend was observed between smoking and mean concentrations of testosterone and LH (Table III). Associations between smoking and mean concentrations of FSH, inhibin B, estrogen and SHBG showed no clear trends. When looking at crude median concentrations, there was also a trend of increased smoking and increased median concentrations of FSH and inhibin B. We calculated the LH/free testosterone ratios [free testosterone = (testosterone/SHBG)\*100] and found increasing LH/free testosterone ratio with more smoking men (data not shown). The median LH/free testosterone ratio among heavily smoking men was 0.09 (p25–75: 0.06–0.13) compared with 0.07 (p25–75: 0.05–0.10) among non-smokers.

### *Smoking and semen quality, subanalysis and stratifications*

The adjusted means in Table III have not been adjusted for BMI, daily coffee consumption or prenatal tobacco smoke

**Table II.** Characteristics of 2542 participants stratified by level of current smoking

Characteristics	Current smoking			
	Non-smoker ( <i>n</i> = 1490) <sup>a</sup>	1–10 cigarettes/day ( <i>n</i> = 436) <sup>a</sup>	11–20 cigarettes/day ( <i>n</i> = 522) <sup>a</sup>	>20 cigarettes/day ( <i>n</i> = 94) <sup>a</sup>
Study subpopulation, number (%)				
Planners	279 (67)	58 (14)	60 (15)	18 (4)
Farmers	201 (79)	19 (7)	31 (12)	5 (2)
Gardeners	104 (68)	23 (15)	21 (14)	4 (3)
Metalworkers	69 (51)	33 (24)	31 (23)	3 (2)
Lead workers	289 (60)	55 (11)	111 (23)	30 (6)
Twins	193 (64)	39 (13)	58 (19)	12 (4)
Inuendo	355 (45)	209 (26)	210 (26)	22 (3)
Person-related characteristics				
Age (years)				
Mean (SD), <i>n</i> = 2514	33.4 (8.3)	31.6 (8.7)	33.8 (8.8)	38.6 (10.3)
Diseases in reproductive organs				
Number (%), <i>n</i> = 2524	116 (8)	21 (5)	25 (5)	9 (10)
Coffee consumption, daily				
Number (%), <i>n</i> = 1001	468 (79)	177 (91)	169 (92)	26 (93)
BMI (kg/m <sup>2</sup> )				
Mean (SD), <i>n</i> = 1973	25.3 (3.4)	24.8 (3.4)	25.3 (3.4)	25.3 (3.4)
Maternal smoking during pregnancy				
Number (%), <i>n</i> = 1583				
Exposed	270 (28)	77 (25)	100 (35)	23 (56)
Unexposed	608 (64)	173 (57)	157 (55)	14 (34)
Unknown	73 (8)	55 (18)	29 (10)	4 (10)
Semen and blood-related characteristics				
Season, number (%), <i>n</i> = 2538				
April–September	554 (37)	207 (47)	221 (42)	43 (47)
October–March	935 (63)	229 (53)	300 (58)	49 (53)
Duration of abstinence, days				
Geometric mean (p25–75), <i>n</i> = 2518	3.3 (2.0–5.0)	3.2 (2.0–5.0)	3.3 (2.0–5.0)	3.4 (2.0–5.0)
Minutes from ejaculation to start of analysis				
Geometric mean (p25–75), <i>n</i> = 2487	49 (35–65)	48 (34–60)	49 (33–63)	51 (35–67)
Recent fever, number (%)	190 (13)	67 (15)	67 (13)	10 (11)
Spillage at sampling, number (%)	278 (19)	89 (20)	104 (20)	23 (20)
Blood collected between 0600 and 1200 h				
Number (%), <i>n</i> = 1209	485 (69)	150 (66)	156 (62)	15 (47)

<sup>a</sup>Because of missing information, these numbers of observations only apply to a few of the variables. The actual number of observations in variables with missing data is given in the left column.

exposure. The inclusion of one or more of these covariates in the models where appropriate produced an effect of essentially the same magnitude (data not shown). For example, the adjusted mean sperm concentration was  $54.7 \times 10^6/\text{ml}$  (95% CI: 44–68) among non-smokers and  $43.9 \times 10^6/\text{ml}$  (95% CI: 26–70) among heavily smoking men when also adjusting for BMI, daily coffee consumption and prenatal exposure to tobacco smoke, a 20% (statistically non-significant) decrease. The mean total sperm count was  $152.0 \times 10^6$  (95% CI: 117–193) among non-smokers compared with  $114.3 \times 10^6$  (95% CI: 60–195) when also adjusting for daily coffee consumption and prenatal exposure to tobacco smoke, a 25% (statistically non-significant) decrease. The number of observations was 981 in the sperm concentration model and 825 in the total sperm count model, and the adjusted tests for overall association and the trend tests were statistically insignificant.

On the contrary, the inclusion of prenatal exposure to tobacco smoke in the semen volume model did not weaken the association between increased smoking and decreased semen volume: both the trend test and the test for overall association remained statistically significant (data not shown).

We stratified the participants according to prenatal tobacco smoke exposure (exposed, *n* = 470; unexposed, *n* = 952 and

self-reported unknown exposure status, *n* = 161) and tested for association between current smoking and sperm concentration (Table IV). We had no information on prenatal tobacco smoke exposure for 959 participants. We observed a statistically non-significant tendency towards decreasing median sperm concentration with increased smoking, among the prenatally exposed (*P* = 0.61 for trend), the prenatally unexposed (*P* = 0.09) and the men with unknown prenatal exposure status (*P* = 0.19). There was a statistically non-significant tendency towards higher sperm concentration among non- and light-smoking prenatally unexposed men compared with non- and light-smoking prenatally exposed men. Prenatal exposure to tobacco smoke did not modify the effect of current smoking on sperm concentration. Similar findings were seen with respect to total sperm count.

We tested the association between cumulative smoking dose (pack-year) and sperm concentration in a linear regression model. The effect of cumulative smoking dose on sperm concentration was very modest or non-existing both among current smokers and among ever-smokers (ex-smokers) (data not shown).

Table V summarizes the median sperm concentrations and total sperm counts in the groups of never-smokers, ever-smokers and current smokers. Never-smokers had higher median sperm

**Table III.** Characteristics of semen and reproductive hormones for participants according to level of current smoking

Characteristics	Current smoking				Test for overall association <i>P</i> -value	Test for trend <i>P</i> -value
	Non-smoker ( <i>n</i> = 1490) <sup>a</sup>	1–10 cigarettes/day ( <i>n</i> = 436) <sup>a</sup>	11–20 cigarettes/day ( <i>n</i> = 522) <sup>a</sup>	>20 cigarettes/day ( <i>n</i> = 94) <sup>a</sup>		
Sperm concentration (×10 <sup>6</sup> /ml)						
Median (p25–75)	57.5 (28–98)	53.0 (28–94)	50.0 (27–90)	41.0 (22–82) <sup>b</sup>	0.05	0.02
Back-transformed mean (95% CI)	56.8 (54–60)	54.3 (50–59)	55.0 (51–59)	44.9 (35–57) <sup>b</sup>	0.14	0.08
Adjusted <sup>d,k</sup> mean (95% CI), <i>n</i> = 2481	56.3 (50–63)	54.1 (47–62)	55.6 (48–64)	45.6 (36–57) <sup>b</sup>	0.20	0.16
Semen volume (ml)						
Median (p25–75)	3.1 (2.2–4.5)	3.1 (2.2–4.3)	3.0 (2.0–4.1) <sup>b</sup>	3.0 (2.0–4.3)	0.06	0.02
Back-transformed mean (95% CI), <i>n</i> = 2045	3.2 (3.1–3.3)	3.1 (3.0–3.3)	2.9 (2.8–3.1) <sup>b</sup>	3.0 (2.6–3.4)	0.06	0.01
Adjusted <sup>e,k</sup> mean (95% CI), <i>n</i> = 2005	3.2 (3.0–3.4)	3.1 (2.9–3.4)	3.0 (2.7–3.2) <sup>b</sup>	3.1 (2.7–3.5)	0.02	0.004
Sperm total count (×10 <sup>6</sup> )						
Median (p25–75)	176 (82–329)	182 (80–299)	144 (69–287) <sup>b</sup>	132 (68–219) <sup>b</sup>	0.08	0.003
Back-transformed mean (95% CI), <i>n</i> = 2045	179 (168–190)	177 (159–196)	161 (146–178)	127 (96–163) <sup>b</sup>	0.04	0.01
Adjusted <sup>f,k</sup> mean (95 % CI), <i>n</i> = 2005	169 (148–192)	164 (139–192)	153 (130–180)	120 (87–161) <sup>b</sup>	0.04	0.01
Percentage motile sperm						
Median (p25–75)	50 (26–64)	52 (31–68) <sup>c</sup>	53 (29–66)	41 (21–60)	0.01	0.19
Mean (95% CI), <i>n</i> = 2059	47 (45–48)	49 (47–52) <sup>c</sup>	49 (47–51)	41 (36–46) <sup>b</sup>	0.01	0.51
Adjusted <sup>g,k</sup> mean (95% CI), <i>n</i> = 1985	32 (24–40)	31 (23–39)	30 (22–39)	28 (18–37)	0.12	0.02
Sex hormone concentrations						
Testosterone (nmol/ml)						
Median (p25–75)	16.4 (13–21)	17.0 (13–22)	16.8 (13–22)	19.0 (12–23)	0.12	0.03
Back-transformed mean (95% CI), <i>n</i> = 1812	16.4 (16–17)	17.0 (16–18)	16.9 (16–18)	17.6 (16–20)	0.16	0.04
Adjusted <sup>h,k</sup> mean (95% CI), <i>n</i> = 1103	24.5 (23–26)	25.8 (24–28) <sup>c</sup>	26.0 (24–28) <sup>c</sup>	26.7 (24–30)	0.02	0.001
Estrogen (pmol/l)						
Median (p25–75)	74.0 (59–93)	74.6 (61–95)	75.0 (62–94)	79.5 (64–95)	0.55	0.21
Back-transformed mean (95% CI), <i>n</i> = 1231	74.9 (73–77)	78.6 (75–83)	77.3 (74–80)	79.9 (72–88)	0.23	0.09
Adjusted <sup>h,k</sup> mean (95 % CI), <i>n</i> = 838	78.4 (74–83)	80.0 (75–85)	83.0 (78–86) <sup>c</sup>	80.4 (69–93)	0.26	0.07
SHBG (nmol/l)						
Median (p25–75)	30.0 (22–39)	27.0 (20–35) <sup>b</sup>	29.5 (22–36)	36.4 (25–52) <sup>c</sup>	0.001	0.47
Back-transformed mean (95% CI), <i>n</i> = 1386	30.1 (29–31)	27.7 (26–29) <sup>b</sup>	29.4 (28–31)	35.7 (31–41) <sup>c</sup>	<0.001	0.92
Adjusted <sup>i,k</sup> mean (95 % CI), <i>n</i> = 985	19.6 (18–21)	19.7 (18–22)	20.5 (19–23)	22.1 (18–26)	0.34	0.10
FSH (IU/l)						
Median (p25–75)	3.8 (2.7–5.4)	3.7 (2.6–5.5)	4.2 (3.0–6.0)	4.4 (3.2–5.9)	0.01	0.01
Geometric mean (95% CI), <i>n</i> = 1811	3.9 (3.8–4.0)	3.9 (3.6–4.1)	4.2 (4.0–4.5)	4.4 (3.8–5.2)	0.045	0.01
Adjusted <sup>h,k</sup> mean (95 % CI), <i>n</i> = 1102	3.2 (2.8–3.6)	3.1 (2.7–3.5)	3.3 (2.9–3.8)	3.2 (2.6–4.0)	0.52	0.37
LH (IU/l)						
Median (p25–75)	3.3 (2.5–4.7)	4.0 (2.8–5.3)	3.7 (2.7–5.0)	4.0 (2.7–4.9)	0.001	0.84
Geometric mean (95% CI), <i>n</i> = 1811	3.3 (3.2–3.4)	3.9 (3.7–4.1) <sup>c</sup>	3.7 (3.6–3.9) <sup>c</sup>	3.8 (3.4–4.4) <sup>c</sup>	<0.001	<0.001
Adjusted <sup>h,k</sup> mean (95 % CI), <i>n</i> = 1103	2.4 (2.2–2.6)	2.5 (2.3–2.8)	2.5 (2.2–2.8)	2.7 (2.3–3.2)	0.13	0.04
Inhibin B (pg/ml)						
Median (p25–75)	180.0 (136–233)	184.0 (140–230)	178.5 (137–221)	200.0 (141–260)	0.46	<0.001
Back-transformed mean (95% CI), <i>n</i> = 1523	180.0 (175–185)	180.4 (172–189)	179.1 (171–187)	200.6 (178–225)	0.35	0.47
Adjusted <sup>h,k</sup> mean (95 % CI), <i>n</i> = 950	175.4 (161–191)	188.3 (171–207) <sup>c</sup>	180.5 (163–200)	195.1 (163–232)	0.15	0.14

CI, confidence interval; SHBG, sex hormone-binding globulin.

<sup>a</sup>These numbers of observations only apply to the crude sperm concentration. Actual number of observations with regard to the other outcome variables is given in the left column.<sup>b</sup>Statistically significantly lower compared with the non-smoker group.<sup>c</sup>Statistically significantly higher compared with the non-smoker group.<sup>d</sup>Back-transformed means are adjusted for study, age, abstinence time, spillage, fever and diseases in reproductive organs.<sup>e</sup>Back-transformed means are adjusted for study, age, abstinence time and season.<sup>f</sup>Back-transformed means are adjusted for study, age and abstinence time.<sup>g</sup>Back-transformed means are adjusted for study, age, abstinence time and minutes from ejaculation to analysis.<sup>h</sup>Back-transformed means are adjusted for study, age and time of day for blood sampling.<sup>i</sup>Back-transformed means are adjusted for study, age, time of day for blood sampling, fever, season and diseases in reproductive organs.<sup>j</sup>Back-transformed means are adjusted for study, age, time of day for blood sampling and season.<sup>k</sup>The following characteristics were chosen as reference in the models: the Planner study; 20 years; 4 days of abstinence; no fever within the last 3 months; sampling between October and March; no diseases in reproductive organs; and blood sampling between 0600 and 1200 h.

concentrations and total sperm counts than current smokers. The median sperm concentration and total sperm count of ever-smokers fell in between the values for never-smokers and current smokers and were not statistically significantly different from either of them. After controlling for age, study, abstinence time, spillage, fever and diseases in the reproductive organs, no statistically significant differences in sperm concentrations were observed among the three groups. Never-smokers

still had a higher total sperm count than current smokers after controlling for study, age and abstinence time.

We repeated the multiple regression analysis for sperm concentration and total sperm count, excluding ever-smokers and men with no information on ever-smoking status from the non-smoking group (data not shown). Essentially, the same results were found, although the difference between non-smokers and heavy smokers was slightly larger. Heavy smokers now had a

**Table IV.** Median (p25–75) sperm concentration among 1583 participants according to level of current smoking stratified by prenatal tobacco exposure status

Prenatal tobacco exposure	Median (p25–75) sperm concentration ( $\times 10^6$ /ml) according to current smoking strata				Test for overall association P-value	Test for trend P-value
	Non-smoker (n = 951)	1–10 cigarettes/day (n = 305)	11–20 cigarettes/day (n = 286)	>20 cigarettes/day (n = 41)		
Exposed, n = 470	57.0 (25–100)	53.0 (25–105)	58.0 (28–105)	50.0 (33–133)	0.88	0.61
Unexposed, n = 952	62.5 (32–102)	59.0 (32–95)	50.0 (26–102)	50.5 (22–94)	0.40	0.09
Unknown exposure status, n = 161	74.0 (42–123)	55.0 (28–110)	73.0 (44–92)	41.0 (16–67)	0.15	0.19

**Table V.** Crude median (p25–75) sperm concentration, median total sperm count and mean (SD) age stratified by ever tobacco smoke exposure status

Ever tobacco smoke exposure status	Age (years), Mean (SD)	Sperm concentration ( $\times 10^6$ /ml), Median (p25–75)	Total sperm count ( $\times 10^6$ ), Median (p25–75)
Never-smoker	32.7 (8.1) (n = 973)	60.0 (30–100) (n = 976)	194 (84–342) (n = 793)
Ex-smoker	33.4 (7.6) (n = 370)	56.0 (31–97) (n = 375)	164 (87–343) (n = 302)
Current smoker	33.3 (9.1) (n = 1039)	50.5 (27–91) <sup>a</sup> (n = 1052)	155 (74–287) <sup>a</sup> (n = 836)

<sup>a</sup>Statistically significantly lower compared with the never-smoker group tested by Wilcoxon-rank sum test.

21% lower sperm concentration and a 31% lower total sperm count than non-smokers (which should be compared with a 19% lower sperm concentration and a 29% lower total sperm count before the exclusion of ever-smokers and men from the non-smoking group for whom no information on ever-smoking status was available).

### OR for oligo- and azoospermia

Thirty-one (1.2%) men had azoospermia and 374 (14.7%) had oligozoospermia (excluding azoospermia). Using logistic regression, we calculated the crude and adjusted ORs for oligozoospermia and azoospermia in relation to current smoking. As depicted in Table VI, we observed no clear trend between increased smoking and increased OR for oligo- or azoospermia. None of the ORs were statistically significantly different from the risk in the reference group of non-smokers.

### Discussion

In this study of >2500 healthy men, we observed a statistically significant dose–response relationship between current cigarette smoking and several semen characteristics. The sperm concentration, the semen volume, the total sperm count and the percentage of motile sperm dropped with increased smoking. Heavily smoking (>20 cigarettes per day) men had ~19%

lower mean sperm concentration and a 29% lower total sperm count than non-smokers after adjustment for differences in other factors related to semen quality, including age, study and abstinence time.

To our knowledge, our study is by far the largest study of ‘normal’ men (i.e. not infertility clients) to investigate the possible dose–response relationship between current smoking and semen quality. The number of participants in the earlier such studies investigating this relationship has not exceeded 900 participants, and most have been much smaller. The most recent and largest study, conducted by Pasqualotto *et al.* (2006), included 522 fertile non-smokers and 367 fertile smokers in three smoking strata. They found a declining semen volume with an increasing number of cigarettes smoked, but no statistically significant differences were observed between the groups in terms of sperm concentration, motility or morphology. In fact, the mean sperm concentration was (statistically non-significantly) higher among smokers of 11–20 cigarettes per day than that among non-smokers ( $125 \times 10^6$ /ml versus  $109 \times 10^6$ /ml). Few other studies also report statistically non-significant trends towards higher sperm concentrations with more smoking, for example the one by Oldereid *et al.* (1989); however, most studies report either a statistically significant dose–response relation between increased smoking and decreased sperm concentration (Lewin *et al.*, 1991; Al Bader *et al.*, 1999)

**Table VI.** Odds ratio (OR) for oligozoospermia (sperm cell concentration >0 and <20  $\times 10^6$ /ml) and azoospermia (0  $\times 10^6$ /ml) in relation to current smoking

	Oligozoospermia			Azoospermia		
	n/N <sup>a</sup> (%)	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)	n/N <sup>c</sup> (%)	Crude OR (95% CI)	Adjusted <sup>b</sup> OR (95% CI)
Non-smoker	212/1467 (15)	Reference	Reference	23/1278 (1.8)	Reference	Reference
1–10 cigarettes/day	62/433 (14)	0.99 (0.73–1.34)	1.02 (0.73–1.41)	3/374 (0.8)	0.44 (0.13–1.48)	0.64 (0.19–2.33)
11–20 cigarettes/day	85/521 (16)	1.15 (0.88–1.52)	1.12 (0.84–1.49)	1/437 (0.2)	0.13 (0.02–0.93)	0.13 (0.02–0.99)
>20 cigarettes/day	15/90 (17)	1.18 (0.67–2.10)	1.09 (0.60–2.00)	4/79 (5.1)	2.91 (0.98–8.63)	2.18 (0.69–6.88)

<sup>a</sup>The number of observations in the crude model is 2511. In the adjusted model, the total number of observations is reduced to 2451.

<sup>b</sup>OR are adjusted for study, age, abstinence time, spillage, fever and diseases in reproductive organs.

<sup>c</sup>The number of observations in the crude model is 2168. In the adjusted model, the total number of observations is reduced to 2001.

or a tendency towards decreased sperm concentration with more smoking (Rodriguez-Rigau *et al.*, 1982; Vogt *et al.*, 1986; Rantala and Koskimies, 1987; Marshburn *et al.*, 1989; Oldereid *et al.*, 1992; Vine *et al.*, 1994; Vine, 1996; Zhang *et al.*, 2000).

We found higher testosterone and LH levels in addition to higher LH/free testosterone ratios with increased smoking. We also observed augmented median FSH and inhibin B levels (not transformed or adjusted) with more smoking. In theory, in the hypothalamo–pituitary–gonadal system, an increase in FSH and LH will initially cause testosterone and inhibin B levels to rise and subsequently induce a decrease in FSH and LH by negative feedback. Our findings suggest that tobacco smoke constituents may disrupt the normal function of this system, and we found evidence suggesting ‘compensated Leydig cell failure’ in smokers. No association between increased smoking and estrogen was found.

Only two of the earlier studies of a possible dose–response relationship between a higher number of cigarettes smoked per day and decreased semen quality have assessed the corresponding reproductive hormone levels: the study by Andersen *et al.* (1984), which included 86 non-smokers, 44 light smokers and 93 medium/heavy smokers, found a positive association between smoking and testosterone, which is in accordance with our results, but the study found no increase in either FSH or LH with more smoking. In the study by Pasqualotto *et al.* (2006) mentioned earlier, no increase in testosterone, FSH or LH was observed.

The following limitations apply to our study. The participation rate was low in some of the original studies (i.e. 16% in the Planner study), which may have introduced a selection bias of unknown direction or magnitude. In the Inuendo study, all the men had proven fertility, and in the Planner study, no one had knowledge of their reproductive potential. In the other studies (the Metalworker study, the Gardener study, the Farmer study, the Lead worker study and the Twin study), some of the participants had knowledge of their reproductive potential. It has been shown that men with reduced fertility are more willing to participate in semen quality studies than other men (Bonde *et al.*, 1996). If this possible selection bias was to explain the results, study participation should be associated both with lower semen quality and with higher degree of smoking, and this seems unlikely, because the overall European smoking prevalence was similar to the smoking prevalence in these studies (Statistics Sweden, 1997).

Information on current smoking was, as all other variables, collected through self-completed questionnaires, which may have introduced a risk of misclassification of the exposure variable. Nevertheless, underreporting of current smoking would have caused the underestimation of the true association.

In the multiple and logistic regression analysis, we controlled for factors that might affect the semen quality and hormonal level. The information collected in each of the studies was not always entirely comparable, which to a certain extent made it difficult to gather the seven data sets into one. We therefore had no valid information on alcohol intake and accordingly could not adjust for this possible confounding factor. As mentioned above, data on BMI, daily coffee consumption and pre-

natal tobacco smoke exposure were missing. This weakened the statistical power when adjustment was made for these covariates, and we therefore did not adjust for these covariates in the initial models (Table III).

Jensen *et al.* have proposed that prenatal exposure to maternal tobacco smoking is a stronger predictor of poor semen quality than current smoking and that the association between current smoking and decreased semen quality may be confounded by the prenatal exposure. Among 1770 young men from five European countries, current smoking had no independent effect on semen quality, whereas prenatally exposed men had ~20% lower sperm concentration and a 25% lower total sperm count compared with prenatally unexposed men (Jensen *et al.*, 2004). Prenatally exposed men have also been reported to have decreased semen quality compared with unexposed men in two other studies (Storgaard *et al.*, 2003; Jensen *et al.*, 2005). In our study, the association between current smoking and decreased semen quality was not confounded by prenatal exposure to maternal smoking. We found essentially the same effect after adjusting for prenatal tobacco smoke exposure, although the number of observations was reduced dramatically, and the trends between increasing current smoking and decreasing sperm concentration and total sperm count became statistically insignificant. We stratified the participants providing information on the prenatal exposure status into three groups (exposed, unexposed and self-reported unknown exposure status). All groups showed a tendency towards decreasing sperm concentration and total sperm count with more smoking (i.e. no effect modification by prenatal exposure status). We observed a statistically non-significant tendency towards lower sperm concentration and total sperm count among prenatally exposed men than those among prenatally unexposed men with similar current smoking status, indicating that prenatal exposure to tobacco smoke may be an independent risk factor for decreased sperm concentration and total sperm count. This could, however, not be conclusively confirmed in these data.

Given our cross-sectional design, we are unable to confirm a causal relationship between current smoking and decreased semen quality, but when we stratified the participants into never-smokers, ever-smokers and current smokers, we found a tendency towards decreasing sperm concentration and total sperm count with increasing ever tobacco smoke exposure. Ever-smokers had sperm concentrations and total sperm counts in between the never-smoking and the currently smoking men, suggesting a harmful and partly irreversible effect of adult tobacco smoking. In two very small case-series studies, men were followed for up to 12 months after smoking cessation, and both the studies reported a marked improvement of the semen quality (Schirren and Gey, 1969; Sofikitis *et al.*, 1995). Naturally, large-scale, prospective studies with appropriate control groups have to be conducted to confirm this hypothesis of irreversibility.

Our currently non-smoking group contained both never-smokers and ever-smokers, and it can be argued that the latter should be excluded from the analysis as they had been exposed to cigarette smoke at some time in their adult life. When we excluded ever-smokers and men with no information on ever-smoking from our group of non-smoking men, the difference in sperm

concentration and total sperm count between heavily smoking and non-smoking men rose a few percentage.

The mechanism behind the harmful effect of smoking on semen quality is not fully understood. Disturbance of the hypothalamo–pituitary–gonadal system (Vermeulen, 1993) or mild hypoxia caused by the disruption of the testicular micro-circulation (Collin *et al.*, 1995) are possible explanations, but a direct toxic effect of the many chemical components in the cigarette smoke on the germinative epithelium is a more likely explanation (Zenzes, 2000). Oxidants in cigarette smoke are thought to damage sperm DNA, and smokers have more oxidative DNA damage in their sperm than do non-smokers (Shen *et al.*, 1997; Zenzes *et al.*, 1999; Horak *et al.*, 2003). An association between cigarette smoking and sperm aneuploidy has also been observed (Harkonen *et al.*, 1999; Shi *et al.*, 2001).

In our study, 16% had semen concentrations below  $20 \times 10^6$ /ml, and there was no association between more smoking and heightened odds for oligozoospermia or azoospermia. The numbers were small, and this result should therefore be assessed with caution. In two other Danish studies conducted in the 1990s, the part of men with oligozoospermia was 25% (Andersen *et al.*, 2000) and 17% (Jensen *et al.*, 2000), which is a little larger than that found in the present study.

There is a strong correlation between semen quality and fecundity (Zinaman *et al.*, 2000), and fecundity is reported to rise with sperm concentrations up to  $\sim 40 \times 10^6$ /ml (Bonde *et al.*, 1998a). The median sperm concentration among heavily smoking men in our study was  $41.0 \times 10^6$ /ml (p25–75: 22–82). Another large Danish investigation observed a dose–response relation between male smoking and fecundity (Olsen, 1991), indicating that the reduced semen quality among smokers may have serious consequences for male fecundity.

In conclusion, we found that tobacco smoking in adult life impairs semen quality moderately and independently of prenatal exposure to tobacco smoke. The results seem not to be explained by confounding, selection or information bias. It would be sensible to advise men to abstain from smoking to avoid decreased fecundity.

## Acknowledgements

We thank Henrik Hjøllund, Lone Storgaard, Tina K. Jensen, the Asclepios group (Pietro Apostoli and Michael Joffe in particular) and the Inuendo group (Lars Hagmar, Jan K. Ludwicki, Henning S. Pedersen, Anna Rignell-Hydbom and Valentina Zvezday in particular) for their work in planning and conducting the original semen quality studies. The study was supported by the Health Insurance Foundation (grant number 2004B137 and 2005B081), the Danish Medical Research Council (grant number 22-03-0200, 22-04-0271 and 271-05-0760), the Augustinus Foundation (grant number 05-2620), the Knud Højgaard Foundation (grant number 37.065), the Simon Fougner Hartmanns Family Foundation, the University of Aarhus Research Foundation and the Biomedical Laboratory Scientist Education and Research Fund.

## References

Abell A, Ernst E and Bonde JP (2000) Semen quality and sexual hormones in greenhouse workers. *Scand J Work Environ Health* 26,492–500.  
 Al Bader A, Omu AE and Dashti H (1999) Chronic cadmium toxicity to sperm of heavy cigarette smokers: immunomodulation by zinc. *Arch Androl* 43,135–140.

Andersen AG, Jensen TK, Carlsen E, Jorgensen N, Andersson AM, Krarup T, Keiding N and Skakkebaek NE (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 15,366–372.  
 Andersen AN, Semczuk M and Tabor A (1984) Prolactin and pituitary–gonadal function in cigarette smoking infertile patients. *Andrologia* 16,391–396.  
 Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, Matribi SE, Gony B, Keskes L, Kolbezen M *et al.* (2000) Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a work-shop involving ten laboratories. *Hum Reprod* 15,2360–2368.  
 Bonde JP (1990) Semen quality and sex hormones among mild steel and stainless steel welders: a cross sectional study. *Br J Ind Med* 47,508–514.  
 Bonde JP, Ernst E, Jensen TK, Hjøllund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J and Skakkebaek NE (1998a) Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 352,1172–1177.  
 Bonde JP, Giwercman A and Ernst E (1996) Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. *Occup Environ Med* 53,511–519.  
 Bonde JP, Hjøllund NH, Jensen TK, Ernst E, Kolstad H, Henriksen TB, Giwercman A, Skakkebaek NE, Andersson A and Olsen J (1998b) A follow-up study of environmental and biologic determinants of fertility among 430 Danish first-pregnancy planners: design and methods. *Reprod Toxicol* 12,19–27.  
 Bonde JP, Joffe M, Apostoli P, Dale A, Kiss P, Spano M, Caruso F, Giwercman A, Bisanti L, Porru S *et al.* (2002) Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occup Environ Med* 59,234–242.  
 Chia SE, Ong CN and Tsakok FM (1994a) Effects of cigarette smoking on human semen quality. *Arch Androl* 33,163–168.  
 Chia SE, Xu B, Ong CN, Tsakok FM and Lee ST (1994b) Effect of cadmium and cigarette smoking on human semen quality. *Int J Fertil Menopausal Stud* 39,292–298.  
 Collin O, Kilter S and Bergh A (1995) Tobacco smoke disrupts testicular microcirculation in the rat. *Int J Androl* 18,141–145.  
 Evans HJ, Fletcher J, Torrance M and Hargreave TB (1981) Sperm abnormalities and cigarette smoking. *Lancet* 1,627–629.  
 Harkonen K, Viitanen T, Larsen SB, Bonde JP and Lahdetie J (1999) Aneuploidy in sperm and exposure to fungicides and lifestyle factors. ASCLEPIOS. A European concerted action on occupational hazards to male reproductive capability. *Environ Mol Mutagen* 34,39–46.  
 Horak S, Polanska J and Widlak P (2003) Bulky DNA adducts in human sperm: relationship with fertility, semen quality, smoking, and environmental factors. *Mutat Res* 537,53–65.  
 Jensen TK, Jorgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus O *et al.* (2004) Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. *Am J Epidemiol* 159,49–58.  
 Jensen MS, Mabeck LM, Toft G, Thulstrup AM and Bonde JP (2005) Lower sperm counts following prenatal tobacco exposure. *Hum Reprod* 20,2559–2566.  
 Jensen TK, Vierula M, Hjøllund NH, Saaranen M, Scheike T, Saarikoski S, Suominen J, Keiski A, Toppari J and Skakkebaek NE (2000) Semen quality among Danish and Finnish men attempting to conceive. The Danish First Pregnancy Planner Study Team. *Eur J Endocrinol* 142,47–52.  
 Kunzle R, Mueller MD, Hanggi W, Birkhauser MH, Drescher H and Bersinger NA (2003) Semen quality of male smokers and nonsmokers in infertile couples. *Fertil Steril* 79,287–291.  
 Larsen SB, Spano M, Giwercman A and Bonde JP (1999) Semen quality and sex hormones among organic and traditional Danish farmers. ASCLEPIOS Study Group. *Occup Environ Med* 56,139–144.  
 Lewin A, Gonen O, Orvieto R and Schenker JG (1991) Effect of smoking on concentration, motility and zona-free hamster test on human sperm. *Arch Androl* 27,51–54.  
 Mahmoud AM, Depoorter B, Piens N and Comhaire FH (1997) The performance of 10 different methods for the estimation of sperm concentration. *Fertil Steril* 68,340–345.  
 Makler A, Zaidise I, Paldi E and Brandes JM (1979) Factors affecting sperm motility. I. In vitro change in motility with time after ejaculation. *Fertil Steril* 31,147–154.  
 Marinelli D, Gaspari L, Pedotti P and Taioli E (2004) Mini-review of studies on the effect of smoking and drinking habits on semen parameters. *Int J Hyg Environ Health* 207,185–192.  
 Marshburn PB, Sloan CS and Hammond MG (1989) Semen quality and association with coffee drinking, cigarette smoking, and ethanol consumption. *Fertil Steril* 52,162–165.



- Merino G, Lira SC and Martinez-Chequer JC (1998) Effects of cigarette smoking on semen characteristics of a population in Mexico. *Arch Androl* 41,11–15.
- Oldereid NB, Rui H, Clausen OP and Purvis K (1989) Cigarette smoking and human sperm quality assessed by laser-Doppler spectroscopy and DNA flow cytometry. *J Reprod Fertil* 86,731–736.
- Oldereid NB, Rui H and Purvis K (1992) Lifestyles of men in barren couples and their relationships to sperm quality. *Eur J Obstet Gynecol Reprod Biol* 43,51–57.
- Olsen J (1991) Cigarette smoking, tea and coffee drinking, and subfecundity. *Am J Epidemiol* 133,734–739.
- Osser S, Beckman-Ramirez A and Liedholm P (1992) Semen quality of smoking and non-smoking men in infertile couples in a Swedish population. *Acta Obstet Gynecol Scand* 71,215–218.
- Pacifici R, Altieri I, Gandini L, Lenzi A, Pichini S, Rosa M, Zuccaro P and Dondero F (1993) Nicotine, cotinine, and trans-3-hydroxycotinine levels in seminal plasma of smokers: effects on sperm parameters. *Ther Drug Monit* 15,358–363.
- Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB and Lucon AM (2006) Cigarette smoking is related to a decrease in semen volume in a population of fertile men. *BJU Int* 97,324–326.
- Rantala ML and Koskimies AI (1987) Semen quality of infertile couples – comparison between smokers and non-smokers. *Andrologia* 19,42–46.
- Rodriguez-Rigau LJ, Smith KD and Steinberger E (1982) Cigarette smoking and semen quality. *Fertil Steril* 38,115–116.
- Saaranen M, Suonio S, Kauhanen O and Saarikoski S (1987) Cigarette smoking and semen quality in men of reproductive age. *Andrologia* 19,670–676.
- Schirren C and Gey G (1969) The effect of smoking on the reproductive ability in man and woman. *Z Haut Geschlechtskr* 44,175–182.
- Shen HM, Chia SE, Ni ZY, New AL, Lee BL and Ong CN (1997) Detection of oxidative DNA damage in human sperm and the association with cigarette smoking. *Reprod Toxicol* 11,675–680.
- Shi Q, Ko E, Barclay L, Hoang T, Rademaker A and Martin R (2001) Cigarette smoking and aneuploidy in human sperm. *Mol Reprod Dev* 59,417–421.
- Sofikitis N, Miyagawa I, Dimitriadis D, Zavos P, Sikka S and Hellstrom W (1995) Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. *J Urol* 154,1030–1034.
- Sofikitis N, Takenaka M, Kanakas N, Papadopoulos H, Yamamoto Y, Drakakis P and Miyagawa I (2000) Effects of cotinine on sperm motility, membrane function, and fertilizing capacity in vitro. *Urol Res* 28,370–375.
- Spira A, Ducot B, Jouannet P, Soumah A, Feneux P and Albert M (1981) Consommation de tabac, d'alcool, et fertilité masculine. In Spira A and Jouannet P (eds) *Facteurs de la fertilité humaine/Human fertility factors*. Vol. 103. Inserm, Paris, pp. 363–378.
- Statistics Sweden (1997) *Tobacco Consumption 1970–1994 in the Member States of the EU, in Norway & Iceland*. Statistics Sweden, Stockholm.
- Storgaard L, Bonde JP, Ernst E, Andersen CY, Kyvik KO and Olsen J (2002) Effect of prenatal exposure to oestrogen on quality of semen: comparison of twins and singleton brothers. *BMJ* 325,252–253.
- Storgaard L, Bonde JP, Ernst E, Spano M, Andersen CY, Frydenberg M and Olsen J (2003) Does smoking during pregnancy affect sons' sperm counts? *Epidemiology* 14,278–286.
- Toft G, Axmon A, Giwercman A, Thulstrup AM, Rignell-Hydbom A, Pedersen HS, Ludwicki JK, Zvyetzday V, Zinchuk A, Spano M *et al.* (2005) Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross-sectional study. *Environ Health* 4,26.
- Vermeulen A (1993) Environment, human reproduction, menopause, and andropause. *Environ Health Perspect* 101,91–100.
- Vine MF (1996) Smoking and male reproduction: a review. *Int J Androl* 19,323–337.
- Vine MF, Margolin BH, Morrison HI and Hulka BS (1994) Cigarette smoking and sperm density: a meta-analysis. *Fertil Steril* 61,35–43.
- Vine MF, Tse CK, Hu P and Truong KY (1996) Cigarette smoking and semen quality. *Fertil Steril* 65,835–842.
- Vogt HJ, Heller WD and Borelli S (1986) Sperm quality of healthy smokers, ex-smokers, and never-smokers. *Fertil Steril* 45,106–110.
- Wang SL, Wang XR, Chia SE, Shen HM, Song L, Xing HX, Chen HY and Ong CN (2001) A study on occupational exposure to petrochemicals and smoking on seminal quality. *J Androl* 22,73–78.
- Wong WY, Thomas CM, Merkus HM, Zielhuis GA, Doesburg WH and Steegers-Theunissen RP (2000) Cigarette smoking and the risk of male factor subfertility: minor association between cotinine in seminal plasma and semen morphology. *Fertil Steril* 74,930–935.
- World Health Organization (1999) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn. Cambridge University Press, Cambridge.
- Zenzes MT (2000) Smoking and reproduction: gene damage to human gametes and embryos. *Hum Reprod Update* 6,122–131.
- Zenzes MT, Bielecki R and Reed TE (1999) Detection of benzo(a)pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. *Fertil Steril* 72,330–335.
- Zhang JP, Meng QY, Wang Q, Zhang LJ, Mao YL and Sun ZX (2000) Effect of smoking on semen quality of infertile men in Shandong, China. *Asian J Androl* 2,143–146.
- Zinaman MJ, Brown CC, Selevan SG and Clegg ED (2000) Semen quality and human fertility: a prospective study with healthy couples. *J Androl* 21,145–153.

Submitted on June 7, 2006; resubmitted on August 11, 2006; accepted on August 16, 2006