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The impact of cigarette smoking on human semen parameters and hormones

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BACKGROUND: In this prospective study, semen parameters and hormone concentrations of infertile smokers were compared with infertile non- and ex-smokers. We also determined how many men with idiopathic infertility would stop smoking in an attempt to improve their fertility. METHODS: 1104 men (517 non-smokers, 109 ex-smokers and 478 smokers) with infertility for at least 1 year were evaluated. Evaluation included medical history, physical examination, hormone analysis and two semen analyses. Prior to the second semen analysis, smokers were urged to quit smoking. RESULTS: Smokers were significantly younger ($P < 0.001$), had significantly more round cells in their ejaculates ($P = 0.003$), and the percentage of ejaculates with $>1 \times 10^6$/ml leukocytes was higher in smokers ($P < 0.001$). Increased free and total serum testosterone ($P < 0.001$) and decreased prolactin levels ($P < 0.001$) were found in smokers. No differences were found between non-smokers and ex-smokers. Only 23.1% of the smokers versus 46% non-smokers ($P < 0.001$) returned for a second semen analysis, 14 of whom reduced and 15 of whom quit smoking completely. Testosterone levels were significantly lower in those who were able to stop or reduce smoking ($P < 0.001$). CONCLUSIONS: Smoking does not affect conventional semen parameters, but significantly increases round cells and leukocytes. Only a few idiopathic infertile smokers were able to quit smoking.

Key words: hormones/human semen/male fertility/smoking/withdrawal

Introduction

Despite worldwide anti-smoking campaigns, cigarette smoking is very common. The highest prevalence of smoking is observed in young adult males during their reproductive period (46% smokers between 20 and 39 years) (Langgassner, 1999).

About 30% of the Austrian male population aged 15 and older are smokers. Smoking among men is increasing in Central and Eastern Europe. Overall 35% of European men smoke, with a prevalence of 44% or even higher in the Eastern parts (Bulgaria, Greece, Turkey) and 30% in the Western parts (UK, Sweden, Finland) of Europe (Corrao *et al*., 2000).

Cigarette smoking may be associated with sub-fertility in males and may result in decreased sperm concentration, lower sperm motility, and a reduced percentage of morphologically normal sperm respectively (Lewin *et al*., 1991; Sofikitis *et al*., 1995; Zinaman *et al*., 2000).

Nineteen studies evaluating the influence of smoking on semen parameters in infertile men and nine studies in fertile men have been published so far (Vine, 1996; Zinaman *et al*., 2000). The major shortcoming of these studies is a small overall patient number (only two studies included >500 men, and >200 smokers) (Dikshit *et al*., 1987; Lewin *et al*., 1991).

In a recent meta-analysis (Vine, 1996), including 27 studies on the association between cigarette smoking and semen quality, a mean reduction in sperm concentration of 13%, a mean reduction of sperm motility of 10%, and a mean reduction of morphologically normal sperm of 3% was reported in smokers. Most of the studies, however, which reported a significant difference in semen quality were performed in normal, non-infertility clinic men. Unfortunately, in 25 out of 27 studies in this meta-analysis, the number of smokers was <200 men. Another major shortcoming is the lack of accurate smoking dose information.

Smoking may cause sub-fertility by influencing hormone levels (Vogt *et al*., 1986). Testosterone levels may be unchanged, elevated, or decreased and estradiol levels are mainly found to be elevated in smokers (Vine, 1996).

Smoking may impact on fertility, as reported in a recent study enrolling 200 men (Zinaman *et al*., 2000). In this study it was noted that cigarette smoking was significantly associated with a decreased pregnancy rate and impaired semen parameters. Men with azoospermia were excluded and the authors did not report men with genital disease. In this study only 6% ($n = 12$) were smokers. Although there were only six smokers in both the pregnant and the non-pregnant group, a statistical significance ($P = 0.02$) was calculated.

In order to overcome the shortcomings identified in other studies (i.e. low participant number, and lack of smoking dose

data), we compared semen parameters and hormone levels of a large number of infertile smokers with non-smokers and ex-smokers and evaluated the smoking dose.

It was recently concluded (Vine, 1996), that men with marginal semen quality who wish to have children might benefit from stopping smoking. In addition, there are only limited data on whether men would stop smoking for the prospect of recovering from infertility (Pusch *et al*., 1989). Therefore, we determined how many men would stop smoking if they thought it would increase their fertility.

Materials and methods

Patients

This prospective study was conducted between January 1993 and September 2000 in the infertility unit of the Department of Urology, University of Graz. Men with a history of infertility for at least 1 year, who were able to provide an ejaculate, were consecutively evaluated. Work-up for infertility included a medical history, physical examination, as well as the assessment of hormone and semen parameters.

Medical history and particularly any history of previous genital disease was assessed using a questionnaire including the number of cigarettes per day and the duration of smoking as well as the smoking status of the female partner.

Men who had stopped smoking ⩾6 months prior to the examination for infertility were classified as ex-smokers and men who had never smoked as non-smokers. Every man who had smoked cigarettes for >6 months and was still smoking was classified as a smoker. Smokers were categorized as mild (⩽10 cigarettes per day), moderate (>10 and ⩽20 cigarettes per day) and heavy smokers (>20 cigarettes per day).

Physical examination was performed by a uro-andrologist. All men were examined first in an upright position to rule out the existence of a varicocele. A varicocele was diagnosed by palpating the spermatic cord during the Valsalva manoeuvre. Venous reflux was confirmed by Doppler ultrasound. Any abnormality of the testes, epididymis or ductus deferens was recorded. The testicular size was measured with an orchidometer (Link, Hamburg, Germany) and considered normal if the volume was >14 ml.

Ultrasound of both testes was performed using a linear 7.5 MHz ultrasound probe (Sono Layer-J, Toshiba, Tokyo, Japan or SSD-1700, Aloka, Tokyo, Japan).

Hormone analysis

Sexual hormone analysis included measurement of LH [immunoradiometricassay (IRMA); ICN, High Wycombe, Bucks, UK], FSH (IRMA; ICN), testosterone (radioimmunoassay; Immunotec, Marseille, France) and prolactin (Cobas Core® enzyme immunoassay; Hoffmann–La Roche, Basel, Switzerland) in all men, and in 433 men free testosterone (radioimmunoassay; DPC, Los Angeles, CA, USA), and estradiol (E_2, radioimmunoassay; Immunotec) were measured additionally. All blood samples were drawn between 08.00 and 10.00.

Semen analysis

Semen samples were collected by masturbation in a clean specimen container after a sexual abstinence for 3–6 days, allowed to liquefy and evaluated immediately thereafter according to WHO guidelines (World Health Organization, 1992). Ejaculate volume, fructose, pH, and time to liquefaction were measured. Sperm concentration and the concentration of round cells were determined using a haemocytometer twice per sample (Thoma; Assistent Sondheim/Rhoen, Germany).

In the presence of $>10^6$ round cells/ml these cells were further differentiated using histo-chemical staining to detect peroxidase positive cells (Endtz, 1972). The percentage of peroxidase positive round cells staining brown was determined by counting ⩾100 round cells under the microscope (Axiolab; Carl Zeiss GmbH, Oberkochen, Germany) at 10×40 magnification. Thereafter the concentration of peroxidase positive cells was calculated by multiplying the percentage of peroxidase positive cells by the total concentration of round cells.

Morphology and motility evaluation

For evaluation of sperm morphology, prestained slides (two per semen sample), which are usually used for blood cell differentiation, were smeared with a small volume of semen and allowed to air dry (Testsimplets®; Roche Diagnostics, Mannheim, Germany). Sperm morphology was determined using the WHO criteria (World Health Organization, 1992). Besides the percentage of morphologically abnormal sperm, the sperm head, neck and mid-piece, tail defects, as well as the presence of cytoplasmic droplets were assessed. Multiple defects per spermatozoon were noted, if present, by means of a laboratory cell counter (Clay Adams, Inc., New York, NY, USA) The total number of defects was counted and the teratozoospermic index was calculated (total number of defects/number of sperm with defects).

Motility was determined by evaluating 200 sperm per sample, 60 min after semen collection. Motility was graded as 'a', 'b' or 'c and d' according to the WHO criteria (World Health Organization, 1992).

The results of semen analyses were classified according to the nomenclature of semen variables (World Health Organization, 1992). Normozoospermia was diagnosed when sperm concentration, motility and morphology were within the reference values. The reference value for 'sperm concentration' was $\geqslant 20\times10^6$ sperm/ml, for 'motility' ⩾50% sperm with forward progression (categories 'a' and 'b') or ⩾25% sperm with category 'a' movement, and for 'morphology' ⩾30% sperm with normal morphology respectively. Oligozoospermia was determined when sperm concentration was less than the reference value. Likewise, asthenozoospermia was diagnosed when motility, and teratozoospermia when morphology, were below the reference values. An oligoasthenoteratozoospermia was diagnosed when all three variables (concentration, motility, morphology) were disturbed. Combinations (oligoasthenozoospermia, oligoteratozoospermia and asthenoteratozoospermia) were used when two variables were disturbed. Azoospermia was diagnosed when, even in the sediment after centrifugation at >3000 *g* for 15 min, no sperm were detected.

Immediately after evaluation, all selected variables were entered into a computerized data management system (FileMaker; FileMaker, Inc., Santa Clara, CA, USA).

After the primary evaluation for infertility, all men without a history of, or current, genital disease as well as men who had neither azoospermia nor severe oligozoospermia were invited for a second semen analysis 3 months later. The reason to exclude these men is that men with non-idiopathic infertility received causative treatment as soon as possible, and men with a high grade oligozoospermia or azoospermia were offered assisted reproductive techniques, if appropriate.

Severe oligozoospermia was diagnosed when the sperm concentration was $<5\times10^6$/ml and included men who had oligozoospermia alone or in combination with asthenozoospermia and/or teratozoospermia.

Smokers without any history of, or current, genital disease and smokers who had neither azoospermia nor severe oligozoospermia were informed about the possible adverse effects of cigarette smoking on semen parameters and/or fertility and that no other reason for infertility could be detected. They were advised to stop smoking immediately in order to improve the semen quality and were advised

Table I. Results of semen analyses

	Total $n = 1104$	Non-smokers $n = 517$ (46.8)	Smokers $n = 478$ (43.3)	Ex-smokers $n = 109$ (9.9)
Normozoospermia	404 (36.6)	184 (35.6)	183 (38.3)	37 (33.9)
Asthenozoospermia	206 (18.7)	94 (18.2)	90 (18.8)	22 (20.2)
Oligozoospermia	113 (10.2)	50 (9.7)	51 (10.7)	12 (11.0)
Teratozoospermia	29 (2.6)	15 (2.9)	10 (2.1)	4 (3.7)
Asthenoteratozoospermia	69 (6.3)	34 (6.6)	27 (5.6)	8 (7.3)
Oligoasthenozoospermia	68 (6.2)	32 (6.2)	26 (5.4)	10 (9.2)
Oligoteratozoospermia	20 (1.8)	10 (1.9)	9 (1.9)	1 (0.9)
Oligoasthenoteratozoospermia	95 (8.6)	47 (9.1)	37 (7.7)	11 (10.1)
Azoospermia	100 (9.1)	51 (9.9)	45 (9.4)	4 (3.7)*

Values in parentheses are percentages.
*$P = 0.035$.

to return for a semen analysis 3 months after having stopped smoking. In addition to verbal counselling, written information was mailed to every participant of the study.

The remaining smokers with either genital disease or a sperm concentration of $<5\times10^6$/ml were advised to stop smoking as well.

Statistics

Men were grouped into smokers, ex-smokers and non-smokers. A descriptive analysis of the data was performed and the variables were further analysed with a *t*-test and analysis of variance (ANOVA), or with the Wilcoxon–Mann–Whitney test and the Kruskal–Wallis test (FSH, LH, testosterone, free testosterone, E_2, prolactin) depending on the normality assumption.

Variables were categorized whenever possible and analysed with cross-tables using χ^2-test or Fisher's exact test (results of semen analyses, hormones, female smoking status, distribution of non-smokers, ex-smokers and smokers at follow-up). Multiple comparisons of non-smokers with mild, moderate and heavy smokers were done using ANOVA followed by Dunnett's test (Dunnett, 1955). The comparison of the first and the second semen analysis was done with a paired *t*-test. Statistical analysis was performed by a bio-statistician using SPSS statistical software (SPSS Inc., Chicago, IL, USA).

Results

In all, 1104 men were evaluated for infertility. Of these, 517 were non-smokers, 478 smokers and 109 ex-smokers respectively. None was excluded for having started smoking within the previous 6 months.

No significant differences in the results of semen analyses were seen between non-smokers and smokers, whereas with ex-smokers azoospermia was observed significantly less often ($P = 0.035$). The results of semen analyses are given in Table I.

A total of 426 (38.6%) men presented with a history of, or current, genital disease, the type and percentage of which are listed in Table II. The distribution of genital disease among non-smokers and smokers was the same in both groups; in contrast, ex-smokers had significantly less genital disease ($P = 0.0056$, Table III).

Severe oligozoospermia (sperm concentration $<5\times10^6$/ml) and azoospermia were diagnosed in 232 men (21%) and were found in 104 (20.1%) non-smokers, 23 (21.1%) ex-smokers and 105 (22%) smokers respectively.

Severe oligozoospermia and azoospermia in combination with a genital disease were more common in smokers than in non-smokers and ex-smokers (15.5, 13.2 and 11% respectively). However, the difference between smokers and non-smokers was not statistically significant ($P = 0.241$).

Table II. Number and percentage of men with genital disorders ($n = 426$)

Genital disease	No.	% of total (1104)
Varicocele	199	18.0
Pathology of the epididymis and ductus deferens, history of genital infection	71	6.4
Hypogonadism (testis volume <14 ml)	58	5.3
History of orchidopexy	44	4.0
Cryptorchidism	8	0.7
Testicular cancer (presently found)	5	0.5
History of testicular cancer	20	1.8
Snow-storm in testis ultrasound (testicular microlithiasis)	13	1.2
Others	8	0.7

Table III. Number and percentage of non-smokers, smokers and ex-smokers with or without genital disease

	Genital disease		
	No (%)	Yes (%)	Total
Non-smokers	304 (58.8)	213 (41.2)	517
Smokers	292 (61.1)	186 (38.9)	478
Ex-smokers	82 (75.2)	27 (24.8)*	109
Total	678	426	1104

*$P = 0.0056$.

Mean age, body mass index (BMI), as well as semen and hormone parameters for non-smokers, smokers and ex-smokers are shown in Table IV.

Compared with non- and ex-smokers, smokers were significantly younger ($P < 0.01$), had significantly more round cells in their ejaculates ($P = 0.012$), higher LH ($P = 0.035$), higher testosterone ($P < 0.001$), free testosterone ($P = 0.001$) and had lower prolactin levels ($P < 0.001$). The percentage of ejaculates with $>1\times10^6$/ml peroxidase positive round cells was also significantly higher in smokers than non- or

Table IV. Mean age, body mass index and the results of the semen and hormone analyses of non-smokers, smokers and ex-smokers

	Non-smokers (n = 517)	Smokers (n = 478)	Ex-smokers (n = 109)	P
Age	33.4 (6.4)	31.5 (5.1)[a,b]	33.7 (5.7)	<0.001
Body mass index (kg/m^2)	25.8 (3.5)	25.5 (3.3)	26.5 (3.6)[a,c]	0.031
Volume (ml)	3.6 (1.8)	3.7 (1.8)	3.7 (1.7)	0.803
Liquefaction time (min)	38.9 (36.1)	39.4 (34.4)	38.6 (35.1)	0.874
Fructose (μg/ml)	2758 (1379)	2638 (1242)	2918 (1301)	0.191
Morphologically abnormal (%)	57.8 (17.6)	56.1 (17.9)	57.6 (17.6)	0.207
Head defects (%)	39.1 (16.5)	39.2 (15.7)	38.5 (15.2)	0.924
Mid-piece defects (%)	11.3 (6.5)	11.2 (7.1)	12.3 (7.0)	0.401
Tail defects (%)	14.3 (12.0)	14.2 (9.5)	14.1 (9.2)	0.213
Cytoplasmic droplets (%)	7.5 (4.4)	7.7 (4.3)	7.6 (4.5)	0.800
Teratozoospermic index[d]	1.47 (0.16)	1.48 (0.16)	1.50 (0.17)	0.338
Sperm concentration ($\times 10^6$/ml)	57.9 (70.8)	58.8 (63.9)	59.1 (67.2)	0.730
Grade 'a' motility (%)	24.4 (9.4)	24.8 (8.6)	23.4 (10.7)	0.569
Grade 'b' motility (%)	19.2 (5.1)	19.9 (4.6)	18.4 (4.8)	0.12
Round cells ($\times 10^6$/ml)	2.7 (3.38)	3.5 (5.3)[a,b]	2.6 (3.4)	0.012
FSH (mIE/ml)	7.1 (7.9)	7.5 (9.8)	7.4 (6.3)	0.122
LH (mIE/ml)	4.4 (2.8)	5.1 (3.9)[a,b]	4.8 (3.1)	0.035
Testosterone (ng/ml)	4.2 (2.1)	5.0 (2.7)[a,b]	4.4 (2.5)	< 0.001
Free testosterone (pg/ml)	15.2 (5.3)	17.6 (5.6)[a,b]	14.9 (4.1)	< 0.001
Estradiol (pg/ml)	27.4 (42.8)	26.2 (13.2)	26.0 (11.0)	0.335
Prolactin (ng/ml)	13.1 (7.7)	11.2 (4.9)[a,b]	12.1 (5.5)	< 0.001

Values are means (SD).
[a]Significantly different from non-smokers.
[b]Significantly different from ex-smokers.
[c]Significantly different from smokers.
[d]Teratozoospermic index = total number of defects/number of sperm with defects.

ex-smokers (11.8 versus 13.1 versus 23.5% respectively; $P < 0.001$).

In contrast, ex-smokers had a significantly higher BMI ($P = 0.031$) compared with non-smokers and smokers.

Out of 478 smokers, 124 were classified as mild, 244 as moderate and 110 as heavy smokers. Classifying smokers as mild, moderate and heavy, only BMI (24.9, 25.5, 26.1 kg/m^2; $P = 0.05$), the mean number of cigarettes per day (6.5, 18.5, 32.4; $P < 0.001$) and the duration of smoking (10.5, 12, 13.9 years; $P < 0.001$) were significantly different between mild, moderate, and heavy smokers.

A total of 350 (31.7%) female partners smoked. Out of 478 male smokers, 239 (50%) had female partners who were also smokers, whereas only 91 (17.6%) non-smokers and 20 (22.5%) ex-smokers reported that their female partners were smokers ($P < 0.001$).

Men with any history of, or current, genital disease, azoospermia or severe oligozoospermia were further excluded. Men who refused their approval to return for evaluation after 3 months ($n = 12$) were also excluded, thus leaving 588 eligible men for further evaluation (258 non-smokers, 70 ex-smokers and 260 smokers). Of these, 211 men (36%) returned for a follow-up semen analysis.

Significantly more non-smokers ($n = 119$, 46.1%) and ex-smokers ($n = 32$, 45.7%) returned for the second semen analysis. In contrast, only 60 (23.1%) smokers returned for a follow-up semen analysis ($P < 0.001$), 14 of whom had reduced smoking and 15 had completely stopped. Out of 29 smokers, who had either reduced or stopped smoking, only six (20.7%) men had a smoking partner.

The mean testosterone levels of men who had either stopped or decreased smoking (4.3 ng/ml) were significantly lower compared with smokers who did not stop smoking or did not return for further evaluation (5.0 ng/ml) ($P < 0.001$).

Non-smokers and ex-smokers returned for a second semen analysis after a mean time of 14 weeks. Mean time to second semen analysis for men who had stopped, reduced or continued to smoke was 18, 22 and 43 weeks respectively ($P = 0.148$). No significant differences between the first and second semen analysis were noticed.

Discussion

The percentage of smokers in our study of infertile men was 43.3% and was therefore not different from the Austrian male population between 18 and 50 years, which was reported to be 44.2% during the study period (Langgassner, 1999). Males aged 20–24 years have the highest rate of smoking (47.9%) (Langgassner, 1999). This is in accordance with our finding because smokers seeking work-up for infertility were significantly younger than non- and ex-smokers.

In a recent meta-analysis (Vine, 1996) of 27 studies addressing the association between cigarette smoking and semen quality, it was noted that most of the studies report a significant difference in semen quality were performed in normal, non-infertility clinic men. Seven out of nine studies in fertile and only six out of 19 studies in infertile men reported a statistically significant difference in semen quality. The largest study in this meta-analysis (Lewin *et al*., 1991) included 662 infertile men (382 non-smokers, 280 smokers)

and reported a statistically significant difference in sperm concentration (55 versus 46.9×10^6/ml). However, in our large study on 1104 infertile men, including 478 smokers, no differences with respect to conventional semen parameters (sperm concentration, motility and morphology) between non-smokers and smokers were observed.

A possible involvement of round cells and leukocytes, which were significantly elevated in our study in smokers compared with non-smokers, was also reported in a previous investigation (Close *et al*., 1990). The authors observed a trend ($P = 0.12$) towards higher leukocyte numbers in a small study evaluating the ejaculates of 22 infertile smokers. Significantly elevated leukocytes have also been reported in the peripheral blood of smokers (Parry *et al*., 1997). Cigarette smoking seems to activate bone marrow, and it is speculated that blood leukocytosis contributes to the chronic lung inflammation associated with cigarette smoking (van Eeden and Hogg, 2000). The mechanism, however, which activates leukocytes in the semen of smokers, is unclear.

Leukocytes are the major source of reactive oxygen species (ROS) in the ejaculate (Sharma and Agarwal, 1996). Elevated leukocytes may impair fertility by formation of ROS (Ochsendorf, 1999). ROS are harmful to sperm DNA (Shen *et al*., 1999) and membrane phospholipids (Kim and Parthasarathy, 1998) because of oxidation. The effects of excessive oxidation on sperm function have been suggested as detrimental. The role of ROS, however, and whether ROS concentrations were elevated in the semen of smokers, has not been studied yet.

Wolff found that 20% of men with elevated leukocytes in their ejaculate had genital tract infections (positive cultures) (Wolff, 1995).

The fact that ex-smokers had significantly less genital disease and an equal percentage of men with a sperm count of $<5\times10^6$/ml, suggests that ex-smokers might comprise a special group of infertile men. The evaluation of possible effects of smoking, however, on sperm concentration by comparing smokers to ex-smokers should be done cautiously, if at all.

It was previously reported that smoking is a co-factor together with genital disease such as varicocele, and can impair human semen quality (Klaiber *et al*., 1987). In this small study, smokers with a varicocele had a disproportionately high incidence of oligozoospermia. In our study this observation could not be confirmed. Although we found a higher percentage of smokers with genital disease and a sperm concentration of $<5\times10^6$/ml, statistical significance was not achieved.

Decreased prolactin levels have recently been reported in female smokers (Weigert *et al*., 1999), similar to our findings in male smokers. In a study using the GH3 rat pituitary cell line, it was shown that nicotine can down-regulate prolactin gene expression (Coleman and Bancroft, 1995). This might explain why prolactin is significantly decreased in smokers. In rams it was noted that hypo-prolactinaemia may affect LH secretion and influence testicular function by directly affecting testosterone and semen production (Regisford and Katz, 1993). But rams are seasonal breeders and reacted differently during hypo-prolactinaemic periods in spring and autumn. The impact of decreased prolactin levels on human semen quality, therefore, remains unclear.

Significantly increased, decreased, and unchanged levels of testosterone were reported in previous studies (Vine, 1996). In our group of smokers, testosterone levels were significantly increased, which is in line with the larger studies (Vogt *et al*., 1986; Field *et al*., 1994). The significantly elevated LH in smokers suggests a central activation of Leydig cells, which explains elevated testosterone and free testosterone levels. No dose dependence of cigarettes smoked and duration of smoking on testosterone levels was seen in our study. A possible explanation is that smoking may, over time, lead to a degeneration of Leydig cells, which in turn reduces testosterone production. This hypothesis is supported by a recent study on rats that were exposed to cigarette smoke and showed decreased testosterone levels (Yardimci *et al*., 1997). The histological examination of the rat testes in this study showed fewer and degenerated Leydig cells.

The question, however, of whether smoking increases LH, testosterone and free testosterone by itself or whether men with elevated hormone levels are more prone to becoming addicted to cigarette smoking remains unclear. Men with higher testosterone levels are reportedly more often engaged in health risk behaviour than men with lower levels (Booth *et al*., 1999). In our study, men who were able to reduce or stop smoking, however, had significantly lower testosterone levels upon entering the study compared wiht the whole group of smokers. This might support the hypotheses that a high testosterone level enhances a health risk behaviour such as smoking, and on the other hand might make it easier for those with lower testosterone levels to refrain from smoking.

Cigarette smoke is a cell mutagen and carcinogen and may adversely affect fertility. Every smoker should be encouraged to stop smoking, especially if a pregnancy is planned. Cigarette smoke contains a lot of known toxins, which may have detrimental effects on fertility in both sexes. Simply stopping smoking, however, could prevent the toxins contained in cigarette smoke.

In order to determine the percentage of men willing to reduce or stop smoking for the prospect of improved fertility, only men with idiopathic infertility and a sperm concentration of $>5\times10^6$/ml were assessed in our study. The reason for this was that men with non-idiopathic infertility received causative treatment, and men with a high-grade oligozoospermia or azoospermia were offered assisted reproductive techniques, if appropriate.

Significantly more non-smokers than smokers returned for a second semen analysis. As a possible explanation, we suspect that only a few smokers quit smoking, and that many of those who did not stop chose not to attend a follow-up semen analysis. In this context, the female smoking status seemed to play an important role, because >79% of the subjects who had either reduced or stopped smoking had non-smoking partners. The number of smokers who quit smoking, however, was surprisingly low in our study. This is in contrast to a previously reported high acceptance of infertile smokers to stop smoking (Pusch *et al*., 1989). In this study, 63% decided to stop smoking, but the number of smokers is not reported.

Significantly more smokers had partners who smoked too. Smoking in female partners has increased during the last decade (Haidinger *et al*., 1998), which might have an even greater influence on the fertility of a couple than male smoking (Bolumar *et al*., 1996; Augood *et al*., 1998). Almost 80% of men who were able to reduce or stop smoking had a non-smoking partner.

In a previous investigation, sperm motility and morphology improved after 6 months of follow-up in nine men who quit smoking (Sofikitis *et al*., 1995). The results of semen analyses after withdrawing or reducing smoking are not reported in detail in our study. They are of limited significance and need to be interpreted with caution because of the small number of men in these groups and the short follow-up. Further studies are needed to investigate the long-term effects of withdrawing from smoking on conventional semen parameters, round cells and leukocytes.

In conclusion, in our large study with a total of 1104 infertile men including 571 non-smokers, 109 ex-smokers and 478 smokers, no significant differences in conventional ejaculate parameters (sperm concentration, morphology and motility) between non-smokers, ex-smokers and smokers were observed, although azoospermia was more prevalent among ex-smokers than the other two groups. Round cells and leukocytes were significantly increased in the ejaculates of smokers compared with non- and ex-smokers. Since leukocytes generate ROS, this may contribute to infertility in smokers.

We also observed elevated serum levels of testosterone, free testosterone, LH and decreased prolactin levels in smokers, but the mechanism(s) of these changes, if any, remains unclear.

Finally, only a few idiopathic infertile smokers were able to quit smoking.

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