Effect of smoking on semen parameters of men attending an infertility clinic

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Summary

Objective: To assess the effects of smoking on total sperm count, progressive sperm motility and sperm morphology among couples attending an infertility clinic.

Methods: A total of 223 sperm samples (126 smokers and 97 nonsmokers) from men attending an infertility clinic for routine infertility workup were compared on the basis of standard semen analysis.

Results: Cigarette smoking is negatively correlated with progressive motile sperm count ($r = -0.1464$, $p = 0.042$), but not with sperm concentration ($p = 0.961$), total motile sperm count ($p = 0.890$) and sperm morphology ($p = 0.838$). Furthermore, packages/year (cumulative dose of cigarettes) did not correlate with any of the sperm parameters including sperm density ($p = 0.976$), total ($p = 0.559$) and progressive ($p = 0.406$) motile sperm count and sperm morphology ($p = 0.449$).

Conclusions: Although the effect of smoking on male infertility remains inconclusive, smoking had an adverse effect on the progressive sperm motility, irrespective of total amount of cigarettes smoked per day.

Key words: Smoking; Semen analysis; Male infertility.

Introduction

Numerous studies in the literature have not conclusively demonstrated that smoking decreases male fertility [1-3]. This issue is difficult to discern based on the previous publications.

Tobacco smoking is recognized as a general health hazard, and evidence indicates that in both men and women, cigarette smoking affects reproductive health more than the consumption of caffeine or alcohol does [4]. Tobacco smoking yields about 4,000 chemical compounds. In the gaseous fraction, carbon monoxide, nitrogen oxide, ammonia, and hydrocarbons are found, whereas the main component of the particulate phase is composed of aggregates of nicotine. Polycyclic aromatic hydrocarbons activate a proapoptotic protein in mice [5], which leads to damage of oocytes and to reduced fertility. Given the fact that cigarette smoke contains more than 30 chemical agents known to be mutagens, aneugens, or carcinogens in model systems, it is conceivable that there are direct deleterious effects on human embryos and female and male germ cells [6].

The mechanisms by which tobacco smoke affects spermatozoa are poorly understood. The effect of cigarette smoke on sperm function was demonstrated almost two decades ago [7] and has been confirmed by various investigators using classic microscopic analysis [8-11] or computer-assisted sperm analysis, which provides detailed information on sperm kinetics [12]. Some of the studies focused on the relation between cigarette smoking and conventional sperm parameters such as density, progressive motility, and morphology, whose results were conflicting [8, 9, 13-15]. This inconsistency in the literature, leading to a inconclusive results, appears to originate from small samples enrolled in those studies, the differences in the thresholds of daily smoked cigarettes used to distinguish smokers from non-smokers, and the presence of some confounding factors such as partner's smoking habits and alcohol consumption [8, 9].

This retrospective study was aimed at elucidating the impact of smoking on different semen parameters such as sperm count, motility and morphology among well-defined groups of men attending the the infertility unit and who were smokers or non-smokers, respectively (the criteria of smoking was > 1 cigarette use/day).

Materials and Methods

Men attending the andrology laboratory between November 2000 and February 2004 for infertility were included. Before semen analysis, a constructed interview was done to obtain information on age, smoking habits, alcohol use (regular, irregular, or total abstinence), and use or abuse of other substances and drugs. Patients were also asked for history of testicular trauma, postpubertal mumps, varicocele, inguinal hernia surgery and cryptorchidism. Also urological examinations were done to detect abnormalities such as hipogonadism, testicular atrophy and varicocele. A total of 223 sperm samples from 126 smokers and 97 nonsmokers were included in the study. Semen samples were collected by ejaculation into a special sterile container after two to seven days of sexual abstinence. Analysis was performed within two hours after collection.

Semen analysis consisted of determination of sample volume, sperm density (concentration), progressive motility and morphology. Standard clinical semen analysis was performed according to World Health Organization criteria [16]. Motility

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was assessed by computer-assisted sperm analysis using the Hamilton-Thorn-Multispecies HTM-IVOS and was based on the principle of negative-phase contrast microscopy. Oligospermic samples were manually analyzed in a Makler chamber by using a light microscope. Morphology was assessed under the microscope after staining with hematoxylin-eosin and orange-G. Packages/year cigarette use was defined as the cumulative number of cigarette packages smoked in a month. Infertility was defined as the inability of couples to conceive following one-year of unprotected sexual intercourse.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 11.0, Chicago, IL, USA). The unpaired Student's t-test and chi square test were used for analysis of patient characteristics. For correlation analysis, Spearman correlation analysis (r_s) was applied. Continuous data were expressed as mean ± standard error of mean (SEM); a p value of < 0.05 was considered statistically significant.

The present study was approved by Osmangazi University Hospital Ethics Committee three months prior to the enrollment of cases.

Results

Infertile patients were composed of 126 (56.50%) smokers and 97 (43.50%) non-smokers. The mean package/year in smokers was 11.02 ± 0.69. Among the non-smoker group, ten cases (10.3%) were ex-smokers. Mean ages of smokers and non-smokers were 31.55 ± 0.72, 31.01 ± 0.63, respectively (p = 0.96).

As presented in Table 1, patient characteristics including regular alcohol consumption (p = 0.78), environmental toxins (heat exposure, chemical exposure) (p = 0.89), postpubertal mumps (p = 0.93), cryptorchidism (p = 0.70), surgery for inguinal hernia (p = 0.70), varicocele (either in history or at physical examination) (p = 0.40), severe febrile illness (p = 0.70) in the history, and atrophic testes (p = 0.82) detected at genital examination in smokers and non-smokers are similar. Statistical analysis could not be performed for cases associated with hydrocele and hypogonadism due to an inadequate number to reveal a robust result.

Smoker and non-smoker infertile patients were indifferent with regard to sperm concentration (p = 0.68), percentage of total (p = 0.68) and progressive (p = 0.10) motile sperm counts, and normal morphology (p = 0.70) (Table 2). Among non-smokers, semen parameters of ex-smokers did not differ from those of the rest (p = 0.45).

Table 1. — *Patient characteristics regarding history and genital examination in smokers and non-smokers.*

Patient characteristics	Smokers n = 126		Non-smokers n = 97		p value
	n	%	n	%	
Regular alcohol consumption	5	3.97	5	5.15	*p = 0.78
Environmental toxins	11	8.73	9	9.28	p = 0.89
Heat exposure	4	3.17	1	1.03	*p = 0.39
Chemical exposure	8	6.35	7	7.22	p = 0.99
Postpubertal mumps	7	5.56	6	6.19	p = 0.93
Cryptorchidism	4	3.17	2	2.06	*p = 0.70
Inguinal hernia	5	3.97	2	2.06	*p = 0.70
Severe febrile illness	5	3.97	2	2.06	*p = 0.70
Varicocele	29	23.02	28	28.87	p = 0.40
Atrophic testis	8	6.35	6	6.19	p = 0.82
Hydrocele	1	0.79	1	1.03	†
Hypogonadism	1	0.79	–		†

* Fisher exact test; † Statistical analysis could not be performed.

Table 2. — *Sperm concentration, motility and morphology of smoker and non-smoker infertile patients.*

Semen parameters	Smokers mean ± SEM	Non-smokers mean ± SEM	Student's t-test
Concentration (million/ml)	54.29 ± 6.05	57.36 ± 4.48	p = 0.68
Total motility (%)	53.74 ± 2.55	55.12 ± 2.15	p = 0.68
Progressive motility (%)	27.95 ± 2.58	22.73 ± 1.94	p = 0.10
*Normal morphology	11.85 ± 1.22	12.44 ± 0.94	p = 0.70

*According to Kruger's strict criteria.

Table 3 shows that when partial correlation analysis was applied to control for possible confounding factors (history and physical examination findings) cigarette smoking was negatively correlated with progressive motile sperm count ($r_s = -0.1464$, p = 0.042), but not with sperm concentration (p = 0.961), total motile sperm count (p = 0.890) and sperm morphology (p = 0.838). Also packages/year (dose of cigarettes) did not correlate with any of the sperm parameters.

Table 3. — *Correlation of smoking with semen parameters*.*

Semen parameters	Smoking (yes or no)	Smoking (packages/year)
Sperm concentration	$r_s = -0.0036$, p = 0.961	$r_s = -0.0029$ p = 0.976
Total motilility	$r_s = +0.0101$ p = 0.890	$r_s = +0.5480$ p = 0.559
Progressive motility	$r_s = -0.1464$ p = 0.042	$r_s = +0.0779$ p = 0.406
Normal morphology	$r_s = +0.0149$ p = 0.838	$r_s = -0.0710$ p = 0.449

*Spearman correlation analysis with control of possible confounding factors.

Discussion

Many studies have suggested that smoking is associated with altered semen quality [6, 8, 10]. However, the extent of the deleterious effects are not well known and net impact of smoking on male fertility is difficult to discern [1]. Although sperm concentrations, motility and morphology have been found to be reduced among non-smokers, they often remain within normal range according to most studies [6, 8, 10, 11, 14-18].

Chen *et al.* [19], based on their cross-sectional study of 306 men presenting to the andrology clinic, did not support the evidence that there are statistically significant relationships between sperm parameters and smoking status, although current smokers were found to have lower sperm concentrations.

We studied 223 samples from men attending our andrology laboratory. In crude analysis with the unpaired Student's t-test we found that smoker and non-smoker infertile patients were indifferent in regard to sperm concentration, percentage of total and progressive motile sperm counts, and normal morphology (Table 2). Ozgur *et al.* [23], in a retrospective study of 196 infertile males

with different smoking habits, found that morphologic evaluation revealed better results for the non-smokers than the heavy-smokers in terms of tail anomalies and percent of coiled tails.

Nevertheless, there are also some confounding factors detected in the history and physical examination which have an impact on sperm quality. These include regular alcohol consumption, environmental toxins (heat exposure, chemical exposure), postpubertal mumps, surgery for inguinal hernia, varicocele, severe febrile illness, atrophic testis, hydrocele and hypogonadism. Postpubertal mumps is associated with unilateral (30%) and bilateral orchitis (10%) and testicular atrophy following orchitis is generally inevitable [24]. Cryptorchidism also has deleterious effects on spermatogenesis. Thirty percent of cases with unilateral and 50% of those with bilateral cryptorchidism have oligozoospermia [24]. Postpubertal orchiopexy has no improving effect on spermatogenetic activity. Ductus deferens obstruction is observed in 1% of cases after surgery for inguinal hernia. Severe febrile illness especially affects type-B spermatogonia and impairs semen quality two to three months later [23]. Although the concept that varicocele causes male subfertility and therefore varicocelectomy cures male subfertility has been contended for almost 50 years, the mechanisms by which varicocele could affect fertility have not yet been satisfactorily elucidated [25]. Hence, we performed partial correlation analyses to check for these possible confounding factors and found that cigarette smoking was negatively correlated with progressive motile sperm count (r_s = -0.1464, p = 0.042), but not with sperm concentration, total motile sperm count and sperm morphology. To evaluate probable cumulative effects of cigarette smoking, we used a partial correlation analysis to test the association between packages/year use of smoking and sperm parameters. As a result, this study showed that dose of cigarettes did not correlate with any of the semen parameters, as demonstrated in Table 3.

The mechanisms by which cigarette smoking affects semen quality are not fully understood. Smoke contains several chemical agents, many of which are carcinogenic or mutagenic. These agents affect the production and function of healthy normal sperm via different mechanisms. The fact that nicotine and its water-soluble metabolite cotinine are detectable in the seminal plasma of smokers suggests that other harmful components of tobacco smoke could pass through the blood-testis barrier [26]. Using the immunoperoxidase method, Zenzes *et al.* [10] demonstrated the presence of adducts formed between benzo(a)pyrene and sperm DNA in smokers. Lower motility has been associated with abnormalities in the ultrastructure of the flagellum and the axonemal structures of the sperm tail [24]. Pacifici *et al.* [8] found that total motility of spermatozoa was significantly and negatively correlated with concentrations of cotinine and hydroxycotinine in the seminal plasma. Zavos *et al.* [28] investigated the effect of smoking on the ability of seminal plasma to maintain sperm viability and found that seminal plasma from smokers had a strong detrimental effect on motility of spermatozoa from non-smokers. Washing of sperm from smokers and exposure to seminal plasma from non-smokers restored motility.

There are also studies reporting the presence of a negative impact of cigarette smoking on sperm function, including lower total sperm count, increased abnormal morphology and decreased citrate concentration (increased pH) [6, 29]. Smoking may decrease male fertility through a direct effect on the testes and its ability to produce progressively motile, vital sperm (spermatogenesis). Sperm morphology, total sperm count, and sperm density (concentration) are prognostic for fertility in vivo and in vitro [11, 27, 28, 30]. Sepanik *et al.* [31] has pointed out the fact that smoking, via oxidative stress pathways, results in sperm DNA fragmentation and poor embryo quality, compromising the chance of pregnancy and development of childhood cancer in the smokers's offspring.

To conclude, smoking negatively affects progressive sperm motility without any correlation with cumulative dose of cigarettes. Therefore, on the basis of the aforementioned data, smoking should be regarded as a risk factor for male infertility and should be discouraged in couples with a history of infertility or poor obstetric outcomes such as recurrent pregnancy losses.

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