

Effects of seminal plasma from cigarette smokers on sperm viability and longevity

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Objective: To evaluate the effects of cigarette smoking on the ability of seminal plasma (SP) to maintain sperm viability.

Design: Clinical randomized study. Spermatozoa from cigarette smoking or nonsmoking subjects were reconstituted in SP from smokers and nonsmokers and in modified Ham's F-10 medium, followed by sperm quality assessment during a 48-hour incubation period.

Setting: Andrology Institute of Lexington, Lexington, Kentucky.

Patient(s): Twenty men who had been smoking cigarettes for longer than 3 years (30 cigarettes per day or more) and 20 nonsmokers participated in this study.

Main Outcome Measure(s): Improvement in sperm viability by removal of SP—and associated detrimental factors present in the SP—from smoker subjects.

Result(s): The results obtained indicate that the quality of spermatozoa obtained from nonsmokers was superior to that of smokers. The SP from the two patient groups had a definite effect on their respective sperm quality, i.e., beneficial effects for the nonsmokers, detrimental effects for the smokers. Exposure of spermatozoa from the nonsmokers to SP from the smokers resulted in a significant reduction in sperm viability. However, exposure of spermatozoa from the smokers to SP from the nonsmokers or to Ham's F-10 medium yielded significant improvements in sperm viability.

Conclusion(s): The detrimental effects of smokers' SP on nonsmokers' spermatozoa was prominent and a rather unique phenomenon. The results generated in this study could be of clinical significance since removal of smokers' SP and subsequent reconstitution and incubation in physiological media seems to enhance the viability, longevity, and possibly the fertilizing ability of these spermatozoa for use in various assisted reproductive technologies. (Fertil Steril® 1998;69:425–9. ©1998 by American Society for Reproductive Medicine.)

Key Words: Cigarette smoking, seminal plasma, sperm viability, longevity

A large percentage (>36%) of men of reproductive age in the United States smoke (1, 2). A number of investigators have proposed various detrimental effects of smoking on sperm concentration, sperm motility, and percentage of morphologically normal spermatozoa (1–11). The effect of smoking on human Leydig cell function is controversial despite the reported adverse effects of smoking metabolites on Leydig cell function in animals (2, 11). A review of the literature suggests that the influence of smoking on the ability of men to reproduce may be caused by impaired spermatogenesis secondary to various hormonal alterations (1, 2, 6).

Cigarette smoke contains a large number of substances, including nicotine, carbon monoxide, and recognized carcinogens and mutagens such as radioactive polonium, benzo(a)pyrene, dimethylbenz(a)anthracene, dimethylnitrosamine, naphthalene, and methylnaphthalene (1, 2, 12). Many of these constituents, however, have never been evaluated for toxicity, and therefore the complete contents of cigarettes and cigarette smoke remain unknown.

Inhalation of cigarette smoke, whether through active or passive smoking, leads to absorption of these substances through the pulmonary vasculature and blood-borne circula-

Received July 3, 1997;
revised and accepted
October 6, 1997.

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Lexington, Lexington,
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Presented at the 12th
Annual Meeting of the
European Society of
Human Reproduction and
Embryology, Maastricht, the
Netherlands, June 30–July
3, 1996.

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0015-0282/98/\$19.00
PII S0015-0282(97)00540-2

TABLE 1

Seminal characteristics (unprocessed specimens) assessed in specimens obtained from cigarette smokers and nonsmokers.

Patient group	Seminal characteristic assessed				
	Volume (mL)	Count ($\times 10^6/\text{mL}$)	Motility (%)	Grade (0–4)	HOS (%)
Smokers	2.8 \pm 0.4	54.3 \pm 5.7	58.7 \pm 8.1	2.9 \pm 0.3	60.4 \pm 7.1
Nonsmokers	3.1 \pm 0.5	65.6 \pm 6.1	72.3 \pm 8.3	3.4 \pm 0.4	77.8 \pm 8.3

Note. All values are means \pm SD. HOS = hypoosmotic swelling test (percent of swollen spermatozoa).

tion throughout the body (1). It is also possible that those same substances could end up in the seminal plasma (SP) of smokers via various modes of diffusion and active transport (2). In the current study we evaluated the effects of cigarette smoking on the ability of SP to maintain sperm viability.

MATERIALS AND METHODS

Patient Profile

The participants in the study were 20 men between 25 and 35 years old (mean age \pm SD, 31.3 \pm 2.7 years) who had smoked more than 30 cigarettes per day for longer than 3 years and 20 men between 23 and 36 years old (mean age \pm SD, 32.7 \pm 3.1 years) who had never smoked. Subjects who participated in the study had never had urogenital or serious systemic disease. None of the participants had a history of alcohol abuse, reported any exposure to gonadotoxic substances, or had a varicocele or any other testicular or accessory genital gland pathology (by physical examination). All urinalysis studies were normal.

The wives and partners of the participants were independently questioned and confirmed the reports of their husbands/partners on gonadotoxic exposure and cigarette use. The methodology and protocols employed in this study were approved by the Institutional Review Board of the Andrology Institute of Lexington.

Seminal Specimen Collection

A semen specimen was collected by each participant at sexual intercourse with the use of a condom-shaped semen collection device (Male Factor Pak; ZDL, Inc., Lexington, KY) after 3–4 days of sexual abstinence (13). Semen specimens were assessed according to the World Health Organization (WHO; 14) standards. Specimens were assessed for volume, sperm concentration, percentage and grade of motility, percentage of live and dead spermatozoa, sperm morphometric parameters, and for the sperm response to the hypoosmotic swelling (HOS) test.

Processing of Seminal Specimens

Following seminal collection and assessment, each specimen was divided into 3 aliquots: aliquot 1 was used

as a control (unprocessed); aliquot 2 was centrifuged (400 \times g for 10 minutes) and the pellet was resuspended in an equal volume of SP from the opposite patient group; and aliquot 3 was centrifuged and the recovered spermatozoa were resuspended to its original volume using modified Ham's F-10 medium containing 3% (w/v) serum bovine albumin (SpermPrep medium; ZDL, Inc.). Spermatozoa from all three aliquots were incubated (37°C) for 48 hours and assessed at various intervals for the percentage and grade of motility and for the response to the HOS test. The results obtained were analyzed statistically with the use of General Linear Model procedures and Student's *t*-test, as appropriate (15). A *P* value of <0.05 was considered statistically significant.

RESULTS

There was no statistically significant difference in the mean (\pm SD) age of the participants between group 1 (32.3 \pm 2.7 years) and group 2 (32.7 \pm 3.1 years). The period of cigarette smoking ranged from 3.5 to 16.5 years (mean, 10.5 years). Smokers reported the number of cigarettes they smoked during the year previous to the study. Within the smoker group, the range of individual mean values of cigarettes smoked per day was 35 to 60 (mean of 42.5 cigarettes per day; Table 1).

The results of the seminal parameters assessed between the two patient groups are shown in Table 1. There were no statistically significant differences in the semen volume and sperm concentration between groups 1 and 2 (*P* > 0.05). However, there were statistically significant differences in most sperm qualitative measurements performed following processing of seminal specimens (*P* < 0.05; Tables 2–4). The results indicate that the quality of spermatozoa obtained from nonsmokers was superior to that of smokers (unprocessed specimens).

The magnitude of sperm viability and longevity loss, as measured by sperm motility and sperm membrane integrity, was higher in the unprocessed specimens from the smokers. Exposure of spermatozoa from smokers to SP

TABLE 2

Sperm motility in nonsmokers vs. smokers.

Treatment group	Assessment interval (hours)						
	0	4	8	12	24	36	48
Nonsmoker	72.3 ± 6.2*	74.8 ± 6.	71.3 ± 5.	63.9 ± 5.	49.3 ± 4.	42.8 ± 4.1	36.7 ± 3.8*
Unprocessed sperm		0*	1*	0*	7*	0*	
Smoker SP‡	59.4 ± 7.2	56.7 ± 7.	53.6 ± 7.	41.2 ± 7.	41.2 ± 7.	30.6 ± 6.6	24.0 ± 4.3†
Washed sperm	83.7 ± 5.7*	84.0 ± 5.	82.7 ± 5.	78.8 ± 4.	65.1 ± 4.	53.7 ± 4.0	46.4 ± 3.6
Smoker	58.7 ± 7.6	51.2 ± 6.	44.3 ± 6.	38.2 ± 4.	29.1 ± 4.	20.4 ± 5.3	8.7 ± 3.6
Unprocessed sperm		7	5	7	7		
Nonsmoker SP	69.6 ± 6.1*	67.8 ± 6.	65.7 ± 7.	61.0 ± 6.	45.3 ± 6.	33.8 ± 5.3	26.0 ± 4.5†
Washed sperm	60.1 ± 8.4	58.3 ± 8.	53.3 ± 7.	48.7 ± 7.	42.7 ± 7.	32.0 ± 6.2	26.3 ± 5.0†
		1	2†	0†	1†	0†	

Note. All values are means ± SD. SP = seminal plasma.

* $P < 0.05$ vs. smoker specimens washed with Ham's F-10 medium or spermatozoa exposed to seminal plasma from smokers.

† $P < 0.05$ vs. unprocessed sperm from smokers.

‡ Spermatozoa from nonsmokers was reconstituted in seminal plasma from patients in the smoker group.

§ $P < 0.05$ vs. unprocessed specimens; spermatozoa were exposed to seminal plasma from smokers or nonsmokers and to washed sperm from smokers.

|| Spermatozoa from smokers was reconstituted in seminal plasma from patients in the nonsmoker group.

from nonsmokers yielded significant improvements in sperm quality, viability, and longevity during the 48-hour incubation period (Tables 2–4). Smokers' sperm quality was similar to that of nonsmoker subjects following reconstitution and incubation of smokers' spermatozoa with nonsmokers' SP. However, exposure of nonsmokers' spermatozoa to smokers' SP resulted in detrimental effects to sperm quality, viability, and longevity during the 48-hour incubation period (Tables 2–4).

DISCUSSION

The possible detrimental effects of cigarette smoking on male reproductive performance, and specifically on semen parameters, is of great interest and the available data is quite conclusive (1–7, 9–11, 16). In addition, because of the recent desire to better understand and treat infertility in both men and women, it has become important to assess the possible side effects of cigarette smoking on male reproduction (2, 11).

TABLE 3

Progressive motility (grade 0–4) measurements in nonsmokers and smokers.

Treatment group	Assessment interval (hours)						
	0	4	8	12	24	36	48
Nonsmoker							
Unprocessed sperm	3.4 ± 0.3*	3.5 ± 0.3*	3.5 ± 0.3*	3.3 ± 0.3*	2.7 ± 0.2*	2.4 ± 0.3*	2.0 ± 0.1*
Smoker SP†	3.0 ± 0.4	3.0 ± 0.4	2.8 ± 0.4	2.6 ± 0.3	2.0 ± 0.3†	1.5 ± 0.3†	0.8 ± 0.2
Washed sperm	3.6 ± 1.4*	3.6 ± 0.4*	3.6 ± 0.5*	3.5 ± 0.4*	2.9 ± 0.3*	2.5 ± 0.3*	2.3 ± 0.4*
Smoker							
Unprocessed sperm	2.9 ± 0.4	2.8 ± 0.4	2.5 ± 0.4	2.2 ± 0.4	1.3 ± 0.3	0.9 ± 0.4	0.5 ± 0.2
Nonsmoker SP	3.3 ± 0.4*	3.2 ± 0.3	3.1 ± 0.3†	3.0 ± 0.3†	2.3 ± 0.3†	2.0 ± 0.2†	1.3 ± 0.2§
Washed sperm	3.1 ± 0.4	3.1 ± 0.3	3.0 ± 0.3†	2.8 ± 0.3†	2.3 ± 0.3†	2.0 ± 0.2†	1.4 ± 0.2§

Note. All values are means ± SD. SP = seminal plasma.

* $P < 0.05$ vs. smoker specimens washed with Ham's F-10 medium or spermatozoa exposed to seminal plasma from smokers or nonsmokers.

† $P < 0.05$ vs. unprocessed sperm from smokers.

‡ Spermatozoa from nonsmokers was reconstituted in seminal plasma from patients in the smoker group.

§ $P < 0.05$ vs. unprocessed specimens from smokers and nonsmoker spermatozoa exposed to seminal plasma from smokers.

|| Spermatozoa from patients who smoked was reconstituted in seminal plasma from patients in the nonsmoker group.

TABLE 4

Sperm response to the hypoosmotic swelling test in nonsmokers vs. smokers.

Treatment group	Assessment interval (hours)		
	0	24	48
Nonsmoker			
Unprocessed sperm	77.8 ± 6.1*	68.9 ± 5.5*	40.3 ± 5.0*
Smoker SP†	63.3 ± 7.2	42.5 ± 6.8‡	22.9 ± 3.9‡
Washed sperm	80.1 ± 6.5*	77.5 ± 6.1*	49.7 ± 4.5*
Smoker			
Unprocessed sperm	60.4 ± 8.1	25.7 ± 5.9	9.1 ± 3.7
Nonsmoker SP§	72.8 ± 7.5*	48.9 ± 6.9‡	30.6 ± 5.1‡
Washed sperm	61.8 ± 7.3	43.7 ± 6.8‡	24.6 ± 5.3‡

Note. All values are means ± SD. HOS = hypoosmotic swelling test; SP = seminal plasma.

* $P < 0.05$ vs. smoker sperm washed with Ham's F-10 medium or spermatozoa exposed to SP from smokers.

† Spermatozoa from nonsmokers was reconstituted in SP from patients in the smoker group.

‡ $P < 0.05$ vs. unprocessed specimens from smokers.

§ Spermatozoa from smokers was reconstituted in SP from patients in the nonsmoker group.

Because of the current knowledge concerning other toxins on reproduction, scientists rationalize that male reproduction can be impaired by a small but increasing number of environmental and occupational exposures (17). Chemical agents or mutagens may affect male reproduction via direct effect on the testes and their ability to produce sperm via the process known as spermatogenesis (2, 18, 19). Those mechanisms may involve the hormonal control of spermatogenesis or may directly affect the germ and Sertoli cells within the seminiferous tubules (2, 6, 11, 20, 21).

Although there is some evidence to the contrary, a number of studies have shown higher incidences of abnormally shaped sperm cells as well as decreased motility and sperm concentration in men who smoke (3, 5, 7, 9–11, 22, 23). Furthermore, fluctuations in male hormones (androgens) and other hormones responsible for the regulation of spermatogenesis and sex drive have been documented in male smokers (2, 6).

Most studies evaluating the effect of smoking on the ability of men to reproduce use two methods (1, 2): the ability of the male to achieve pregnancy in relation to smoking habits and the effect of smoking on the standard parameters of semen analysis. Both methods, although they have inherent limitations, seem to be adequate in pointing out the mode and seriousness of the effect of smoking on the male reproductive system.

In the current study, we investigated the effect of smoking on the ability of SP to maintain sperm viability. This was done by measuring the direct effect of SP obtained from smokers and nonsmokers on a number of sperm parameters.

The SP from each of the two patient groups had a definite effect on the sperm quality of the other group, i.e., beneficial effects for the nonsmokers, detrimental effects for the smokers.

The results obtained indicated quite clearly that SP obtained from smokers had detrimental effects on the sperm quality of the nonsmoker group when reconstituting spermatozoa in SP from the smoker group. It is of interest that the opposite was true for SP from nonsmokers, which had beneficial effects on spermatozoa obtained from smokers. Improvements in sperm quality of smokers' spermatozoa reconstituted with SP from nonsmokers was higher than in those same specimens washed with Ham's F-10 medium. It has been suggested that the SP from nonsmokers may contain a protective substance or factor involved in the protection of spermatozoa against cigarette smoke metabolites and that this substance or factors may be decreased or inactivated in the SP of smokers.

The detrimental effects of smokers' SP on nonsmokers' spermatozoa was prominent and a unique phenomenon that was documented for the first time in this study. The results generated in this study could be of clinical significance since removal of smokers' SP and subsequent incubation of spermatozoa in physiological media seems to enhance the viability, longevity, and possibly the fertilizing ability of these spermatozoa for use in various assisted reproductive technologies.

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