

The influence of smoking on male reproductive parameters in a group of Iraqi subfertile men

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Summary:

Background: Among different air pollutants, cigarette smoke contains toxic chemicals, mutagenic and carcinogenic compounds, which can adversely affect male fertility. In this study, semen parameters and reproductive hormonal concentrations of subfertile smokers were compared with subfertile non-smokers.

Objectives: evaluation of the effect of cigarette smoking on male fertility by evaluating several semen parameters as well as some reproductive hormones in a group of smoker and non smokersubfertile Iraqi subjects.

Patients and Methods: At the male infertility clinic of Al-yarmuk teaching hospital, Almustanseria medical college, Baghdad, Iraq from the 1st of October 2010 to the end of June 2011, 88 men (49 non-smokers, and 39 smokers) with history of subfertility for at least 1 year were evaluated by medical history, physical examination, semen analyses and reproductive hormonal profile.

Results: There was a significant impairment of semen parameters namely sperm count, sperm motility and normal sperm morphology in subfertile smokers (means $\pm$ SD were 23 ± 28.01 , 11.25 ± 16.96 and 35.41 ± 26.44 respectively as compared to subfertile non smokers in which means $\pm$ SD were 53.67 ± 22.16 , 27.04 ± 15.70 , 52.04 ± 17.90 respectively and P values were < 0.005 for the 3 parameters with a non significant changes in reproductive hormonal profile between smokers and non smokers.

Conclusion: Smoking does affect conventional semen parameters namely sperm count, motility and morphology significantly with mild non significant changes in male reproductive hormonal analysis.

Keywords: male subfertility, smoking, semen, reproductive hormones.

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Introduction:

Over the past two decades, there has been an increasing body of evidence that several environmental toxicants may impair semen quality and thus male fertility in animals as well as in the human (1). Among these pollutants, cigarette smoke contains toxic chemicals, mutagenic and carcinogenic compounds, which can adversely affect male fertility too (2). Some of these substances (nicotine and its metabolite cotinine, cadmium. . .) were found in seminal plasma at concentrations proportional to those in the serum, suggesting their crossing of the blood-testis barrier (3). Tobacco combustion yields about 4000 compounds; the smoke can be divided into a gaseous phase and a particulate phase. The principal harmful components of the gaseous phase are carbon monoxide, nitrogen oxide, ammonia and volatile hydrocarbons. The main components of the particulate phase are nicotine and cadmium (4). Nicotine is quickly absorbed through the respiratory tract, mouth mucosa and skin. About 80% to 90% of nicotine is metabolized by body organs; mainly by the liver, but also by the kidneys and lungs (5). Tobacco use affects every system involved in the reproductive process and all stages of reproductive functions are targets of cigarette smoke toxicants. (6) Studies have found that sperm damaged by smoking may also result in more couples having baby girls

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than boys. (7) The researchers suggest that the sperm cells carrying the Y chromosome are more vulnerable to the toxins in cigarette smoke. (8). Tobacco effects can be observed at both microscopic and molecular levels. Microscopically, semen volume, sperm concentration, motility and morphology are affected (9), concomitant with a reduced concentration mainly of citrate and also of fructose (10). At the molecular level, an increased risk of sperm aneuploidy (11), higher levels of seminal oxidative stress (12), alteration of sperm plasma membrane phospholipids asymmetry (13) and sperm DNA fragmentation have been documented (14) and (15). In addition, cigarette smoking has been correlated with poor sperm function in sperm penetration assays (16). Also severe alterations in the flagellar ultrastructure of sperm from smokers was reported (17). Mounting evidence shows a significant association between smoking and male sexual impotence with the association increasing with the number of cigarettes smoked per day. (18). The aim of our study was to evaluate the effect of cigarette smoking on male fertility by evaluating several semen parameters as well as some reproductive hormones in a group of subfertile Iraqi subjects.

Patients and methods:

Patients who were evaluated at the infertility clinic of Al-Yarmok teaching hospital between October, 2010 and July,

2011 with a history of subfertility for at least 1 year were eligible for this study. All of them had normal female partners regarding history, physical examination and investigations. Clinical evaluation of all participants included history, genital examination. Scrotal Doppler ultrasound was performed, as needed, to exclude subclinical varicocele. History included data on smoking, alcohol, recreational drugs, fever, and exposure to gonadotoxins such as chemotherapy, radiotherapy, or pesticides. Subfertile patients who are either smokers (in which they smoked cigarettes on a regular basis (at least 20 cigarettes per day for at least 1 year before enrollment in the study) or who were nonsmokers (never smoked before) were included in the study and having a normal genital examination.

Patients with a history of recreational drug use (i.e., marijuana use and/or narcotic agents) or alcohol consumption (including social drinking) within the past year were excluded. Also, patients were excluded if they had a history of a recent fever or exposure to gonadotoxins such as chemotherapy, radiotherapy, or pesticides. Patients who had abnormalities in their genital examination such as cryptorchidism, varicoceles and genital tract infections, were excluded. Based on history and genital examination, the patients ages range between 21 and 58 years were classified into 2 groups: group 1, smokers (n= 39) and group 2 nonsmokers (n= 49).

Semen Analysis: All subjects were required to collect semen specimens by masturbation in a private room near the laboratory after a period of 3 to 5 days of sexual abstinence. Following liquefaction at 37 °C a drop of 20 µl of homogenized semen is placed by a micropipette on a warm clean microscopic glass slide and covered with a cover slip Examination was performed by light microscope under a magnification of 400X as stated by WHO manual for human semen analysis 2010 (19).

Hormonal assay: Venous blood was collected in plain tubes for the preparation of the serum by centrifugation then serum was collected and frozen at -20 degree centigrade in the freezer until hormonal analysis which was performed by using Addendum-Mini VIDAS apparatus (VIDAS) 12 model, 1992, (Biomerieux company, France), through an enzyme linked fluorescent assay (ELFA) technique.

Statistical Analysis: Using SPSS (statistical package of social sciences) V. 14 for Statistical Analysis. Independent sample T test was used to determine the effects of smoking on standard semen variables and reproductive hormonal profiles $P < 0.05$ was considered statistically significant.

Results:

The 88 subfertile men initially evaluated for this study were divided into 2 groups smokers and non smokers, 39 (44.3%) were smokers, their mean age was about 32.77, The remaining 49 (55.7%) were nonsmokers, their mean age was about 33.16. The mean duration of subfertility of the smoker group was 5.3 years and 65% of them report history of less than 5 years subfertility as compared to 38% who report 5-10 years history and only 5% were having history of more than 10 years subfertility. The mean duration of subfertility of the non smokers was 5.1 years and 57% of them report history of less than 5 years subfertility as compared to 29% who report 5-10 years history and only 14% were having history of more than 10 years subfertility in smokers (fig. 1). Regarding the type of subfertility 71% of smokers were having primary subfertility whereas 29% were of secondary type as compared to non smokers in which 39% were having primary subfertility whereas 61% were of secondary type.

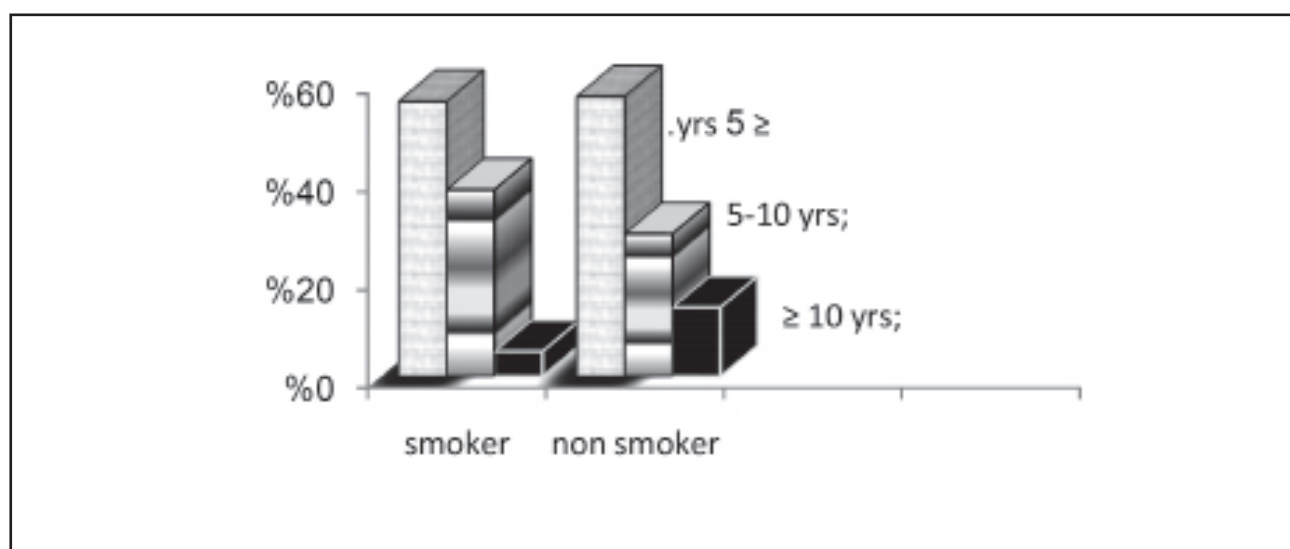


Fig.(1); The distribution of patients according to their duration of subfertility Standard sperm Parameters.

Mean sperm count in non smoker group was about 53.8 as compared to smokers 23.0. On the other hand the mean percent of progressive motile sperms was 27.04% in non smokers as compared to 11.25% in smokers. The mean percentage of normal sperm forms in non smokers was 52.04% whereas that of smokers was 35.42% these three parameters results between the 2 groups (smokers and non smokers were statistically significant (P values were <0.005) as in table 1.

Table 1; Mean semen parameters $\pm$ SD of different semen parameters of smokers and non smokers.

Smoking	Sperm count*	% of progressive motile sperms*	% of normal sperms morphology*
non smoker	53.67 $\pm$ 22.17	27.04 $\pm$ 15.71	52.04 $\pm$ 17.91
Smoker	23.00 $\pm$ 28.01	11.25 $\pm$ 16.96	35.42 $\pm$ 26.44
P value	<0.005	<0.005	<0.005

*P value < 0.005 means statistically significant

Regarding hormonal profile mean serum FSH was lower in non smokers (6.6 mIU/l as compared to smokers (8.5 mIU/l). regarding mean serum LH level it was higher in non smokers (7.5mIU/l) as compared to smokers (4.4 mIU/l). Mean serum estradiol level was higher in non smokers (46.2) pg/ml. as compared to smokers (44.7) pg/ml. Mean serum testosterone level was lower in non smokers (4.06) ng/ml. as compared to smokers (6.01) ng/ml. Mean serum prolactin level was higher in non smokers (12.2) ng/ml. as compared to smokers (9.2) ng/ml. However all these were statistically non significant as in table 2.

Table 2; Mean hormonal profile levels $\pm$ SD of different reproductive hormones of smokers and non smokers.

Smoking	FSH	LH	E2	T	PRL
non smoker	6.62 $\pm$ 6.78	7.50 $\pm$ 19.47	46.27 $\pm$ 14.84	4.06 $\pm$ 1.85	12.29 $\pm$ 5.77
Smoker	8.58 $\pm$ 8.82	4.46 $\pm$ 2.96	44.74 $\pm$ 15.73	6.01 $\pm$ 7.12	9.26 $\pm$ 5.08
P value	0.272	0.355	0.650	0.117	0.098

All were statistically not significant P value > 0.005 means

Discussion:

A causal relationship between cigarette smoking and impaired reproductive function is highly suspected because smokers inhale a host of toxins such as nicotine, carbon monoxide, cadmium, and other mutagenic compounds (9). The link between cigarette smoking and impaired semen

parameters and hence impaired reproductive function may be related to increased levels of seminal reactive oxygen species (ROS) which may be, at least in part, related to the significant increase in leukocyte concentrations in the semen of infertile smokers (20). Because smoking metabolites may induce an inflammatory reaction in the male genital tract with a subsequent release of chemical mediators of inflammation (21). This may overwhelm the antioxidant strategies, resulting in oxidative stress (OS) which in turn impair seminal parameters (22). Another speculation is that toxic metabolites of cigarette smoke may impair spermatogenesis, resulting in the production of defective spermatozoa. In this case, leukocytes infiltrate the male reproductive tract to eliminate defective spermatozoa by phagocytosis (23). In addition to that cigarette smoke itself contains high levels of ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals which all impair seminal parameters (24 and 25). Spermatozoa are particularly susceptible to damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (26) and their cytoplasm contains low concentrations of scavenging enzymes (27–30). In addition, the intracellular antioxidant enzymes cannot protect the plasma membrane that surrounds the acrosome and the tail, forcing spermatozoa to supplement their limited intrinsic antioxidant defenses by depending on the protection afforded by the seminal plasma, which bathes these cells (31). The mechanisms by which cigarette smoking might affect semen quality could not exclude the direct involvement of toxic substances in cigarette smoke, such as nicotine, carbon monoxide, and recognized carcinogens and mutagens, such as radioactive polonium, cadmium, benzo(a)pyrene, and others. Most of these are known to affect male and female gametes and embryos (32). Before a conclusion can be reached as to the potential negative effects of cigarette smoking in infertile men, it is important to bear in mind certain study limitations. In our study, smoking data were obtained from a questionnaire directed to the subjects to determine the number of cigarettes smoked per day and the duration of smoking in years. This was not validated by any biochemical test such as serum or salivary cotinine levels. Although we included infertile smokers who smoked a minimum of 20 cigarettes per day for at least 1 year, we were unable to examine the relationship of smoking with semen parameters in a dose-dependent fashion because of the subjective nature of the smoking history. In addition, our attempts to avoid the potential sources of variability such as alcohol, drugs, and abnormalities of genital examination resulted in a reduction of the total number of smokers in the whole study as well as in each well-defined group. As a result, we were unable to stratify these groups based on the number of cigarettes smoked per day and the years of smoking and adjust for the confounding variables during statistical analysis. Despite these limitations, some firm relationships between cigarette smoking and impaired seminal parameters are evident from our study. We also cannot exclude that our

patients, particularly nonsmoker patients, might have been exposed to secondhand smoke, which can cause immediate harm (34) and, in particular, to prenatal exposure to tobacco smoke, which has been demonstrated to have an adverse effect on semen quality (9). Several studies have reported that the mutagenic components of cigarette smoke adversely affect rapidly dividing cells, including germ cells in the testis (34). Our results agreed with many others who suggested a substantial negative effect of smoking on sperm production, motility, and morphology (9 ; 33 and 35) Because much of the reduced fecundity associated with smoking could be reversed within a year of cessation (33), the clinical significance of the present finding should be to develop effective interventions aimed at helping patients stop smoking for the benefits to general health and for their fertility.

Conclusion:

Although smokers as a group may not experience reduced fertility, males with marginal semen quality may benefit from quitting smoking.

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