

Comparison Study in Seminal Fluid between the Cigarette Smoker and the Non-Smoker among Infertile Males in Erbil City

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Abstract:

Background: Cigarette smoking is a broadly recognized health hazard and a major cause of mortality but still people continue to smoke cigarettes on a regular basis. Tobacco has numerous carcinogens and mutagens which have deleterious effects on human beings. Effect of carcinogens has been observed to be more on rapidly dividing cells which include germ cells. The maximum prevalence of smoking is observed in young adult males in the reproductive period

Aims and objectives: To compare different seminal fluid parameters among smoker and non-smoker, to know the effect of cigarette smoking on sperm counts, motility and morphology, and the difference in seminal fluid variables between smokers and non – smokers.

Patients and Methods: The case - control study performed from May 2015 to December 2015 carried in laboratory of Rizgary Teaching Hospital in Erbil city and a questionnaire prepared for each case. Only patients with primary infertility, who were either smokers or strict non-smokers, were selected and the non - smoker considered as control group.

The selected study group of 100 smokers and 100 strict non - smokers had only one known factor which differentiated them, i.e. cigarette smoking and the duration of smoking was determined by 5 years. A semen specimen was collected after 3days of abstinence period, in a wide – mouth, clean and sterile container. All semen samples were analysed for semen parameters: liquefaction time, volume, viscosity, motility, sperm concentration, morphology and number of pus cells in the sample.

Results: The mean age of both non –smoker and smoker were 33.28 and 34.89 years respectively with maximum and minimum age were 22 and 45 years respectively with the mean duration of infertility for smoker 6.85 years and for the non-smoker was 7.24 years. There were a significant difference in seminal fluid parameters which includes the Ph, viscosity, sperm count, sperm motility, morphology and pus cells in seminal fluid between the smoker and non - smoker while there were no significant difference in volume.

There was significant difference between the two groups regarding seminal fluid variable which includes (Normozoospermia, Asthenozoospermia, Oligoasthenozoospermia, Oligoasthenoteratospermia, Asthenoteratospermia, Teratizoospermia and Oligozoospermia) with p value ($P < 0.000$).

Conclusions: The smoking has bad effect on seminal fluid parameters and there was a difference between the two groups and the smoking can affect and reduce the fertility of the male. Smoking can be a cause of infertility in males especially those with idiopathic infertility.

Key words: Cigarette smoking, Male infertility, Seminal fluid analysis.

Introduction:

Cigarette smoking is a broadly recognized health hazard and a major cause of mortality but still people continue to smoke cigarettes on a regular basis⁽¹⁾.

The World Health Organization⁽²⁾ has reported that approximately one-third of the world's populations older than 15 years are smokers. The maximum prevalence of smoking is observed in young adult males in the reproductive period (46% of smokers are between 20 and 39 years old)⁽³⁾.

The combustion of tobacco 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⁽⁴⁾. Polycyclic aromatic hydrocarbons activate a proapoptotic protein in mice, which leads to damage of oocytes and to reduced fertility. Given that cigarette smoke contains more than 30 chemical agents known to be mutagens, aneugens, or carcinogens in model systems, direct deleterious effects on human embryos and female and male germ cells are plausible⁽⁵⁾.

Infertility can be defined as the incapacity to fulfill pregnancy after a reasonable time of sexual intercourse with no contraceptive measures taken. On the other hand, the World Health Organization definition, based on 24 months of trying to get pregnant, is recommended as the definition that is useful in clinical practice and research among different disciplines⁽⁶⁾.

In (30%) of infertile couples, the male factor, in the form of defective sperm quality, is a major cause⁽⁷⁾. As a large number of men smoke worldwide, and the fact that cigarette smoke contains

known mutagens and carcinogens, there has been much concern that smoking may have unfavorable effects on male reproduction⁽⁸⁾.

Although the general population is well aware of the role of smoking in lung and heart diseases, the undesirable effects of smoking on male reproductive health are less recognized⁽⁹⁾.

Despite this, the undesirable effects of smoking on male reproductive health are less recognized and the impact of cigarette smoking on male fertility and sperm characteristics still remains controversial^(10, 11).

The aims of this study are to:

1. Compare different seminal fluid parameters among smoker and non-smoker.
2. Know the effect of Cigarette smoking on sperm counts, motility and morphology.

Know the difference in seminal fluid variables between smokers and non – smokers.

Patients and Methods:

The case – control study done from May 2015 to December 2015 carried in laboratory of Rizgary teaching hospital in Erbil city and a questionnaire prepared for each case includes: Age, duration of infertility, type of infertility, history of smoking and duration of smoking.

Only patients with primary infertility, who were either smokers or strict non-smokers, were selected and the non – smoker considered as control group.

The following were excluded from the study group:

1. Patients suffering from secondary infertility, as presence of other co-factors may have interfered with our observations.

2. Patients with history of injury to the testes, varicocele, hydrocele, undescended testis or its corrective surgery and vasectomy-reversal surgery.
3. Patients with history of any chronic illness, such as tuberculosis, diabetes mellitus, hypertension, thyroid diseases, mumps or any ailment for which long-term medication was being given.
4. Azoospermics seminal fluid variable.
5. Patients above 45 years of age, to avoid effects of ageing on sperm variables.

Sample size:

Thus, the selected study group of 100 smokers and 100 strict non-smokers had only one known factor which differentiated them, i.e. cigarette smoking and the Duration of smoking was determined by 5 years⁽¹²⁾:

A semen specimen was collected after 3 days of abstinence period, in a wide – mouth, clean and sterile container⁽¹³⁾.

Each patient had been told to urinate before semen collection to decrease contamination of semen from debris or leukocytes⁽¹⁴⁾.

It is important that the entire specimen be collected. Semen was incubated for 30 minutes at 37°C for liquefaction. Name, time of ejaculation and period of abstinence were mentioned⁽¹⁵⁾ and these samples were used for semen analysis.

All semen samples were analysed for semen parameters such as: volume, viscosity, motility, sperm concentration, morphology and number of pus cells in the sample.

A macroscopic and microscopic evaluation of the semen was performed within 30 minutes of liquefaction time after ejaculation at 37°C⁽¹⁶⁾.

The volume of the ejaculate was accurately measured by using a graduated cylinder or a pipette⁽¹⁷⁾ and the time for the semen to liquefaction also measured⁽¹⁸⁾.

All samples were kept at $37 \pm 2^{\circ}\text{C}$ and processed immediately after complete liquefaction.

Following liquefaction of semen, the wet sample was first assessed by placing 10 µl of semen onto a glass slide and covered with a coverslip.

Approximately 200 spermatozoa in 5 fields at 40x magnification were counted for calculating the percentage of motility under four categories as the motility of each spermatozoon graded a, b, c, or d according to whether it shows⁽¹⁷⁾.

a- rapid progressive motility.

b- slow or sluggish motility.

c- non progressive motility.

d- Immotile.

Sperm counting was then done by using modified Neubauer counting chamber after 1 ml of semen diluted with 19 ml of buffer formal saline applied to the outer edges of the cover slip on each side of the chamber using the pasture pipette.

The sperm concentration and the total count were calculated in the following way:

Counted sperm in five small squares X 20 dilution⁽¹⁸⁾.

Sperm morphology was studied on Papanicolaou stained smears, counting a minimum of 200 spermatozoa using 100 × magnification oil-immersion lenses. Sperm vitality was assessed in wet mount smears after supravital staining with Eosin⁽¹⁹⁾.

Table (1): Normal values of semen variables. Cited from (WHO, 1999) ⁽¹⁶⁾.

Standard tests	Normal values
Volume	2.0 ml
PH	7.2-7.8
Sperm concentration	$\geq 20 \times 10^6 / \text{ml}$
Total sperm count	$\geq 40 \times 10^6 / \text{ejaculate}$
Motility	50% or more with forward progression or 25% or more with rapid Progression within 60 minutes of ejaculation.
Morphology	30% or more with normal forms
White blood cells	$\leq 1.0 \times 10^6 / \text{ml}$

Results:

The mean age of both non –smoker and smoker were 33.28 and 34.89 with no significant differences years respectively with maximum and minimum age were 22 and 45 years respectively with the mean duration of infertility for smoker 6.85 years and for the non-smoker was 7.24 years with no significant difference between them. The result of this study showed that there was a difference in seminal fluid

parameters between the smokers and non –smokers as shown in table (2). The result of seminal fluid analysis showed different seminal fluid variables which divided into: (Normozoospermia, Asthenozoospermia, Oligoasthenozoospermia, Oligoasthenozoospermia, Oligoasthenozoospermia, Teratozoospermia and Oligozoospermia) showed a difference between the smokers and non- smokers groups, and there was high significant difference statistically between the two groups as shown in table (3) and figure(2).

Table (2): The difference in some seminal fluid parameters between smokers and non-smoker

seminal fluid parameters	smoker		Non -smoker		P value	statistical analysis
	mean	St. deviation	mean	St. deviation		
Volume of semen	2.725	1.5119	2.486	1.1798	0.082	NS
ph of semen	7.453	0.4838	7.479	0.3666	0.026	S
Vscocity of semen	1.16	0.545	1.08	0.394	0.017	S
Count of sperms	116.186	75.5118	130.138	83.349	0.174	NS
Normal form of sperms	56.43	23.583	66.13	18.233	0	HS
Abnormal form of sperms	43.35	22.873	33.65	17.666	0	HS
Total motility of sperms	63.83	26.192	71.76	23.131	0.002	HS
%grade a	22.86	13.1095	25.5803	11.17122	0	HS
%grade b	21.55	10.77162	25.2902	9.75227	0.004	S
%grade c	19.06	7.07652	19.691	8.30447	0.41	NS
%grade d	37.02	26.09771	29.16	23.29769	0.005	HS
Pus in seminal fluid	4.3	4.345	2.51	3.672	0.01	S

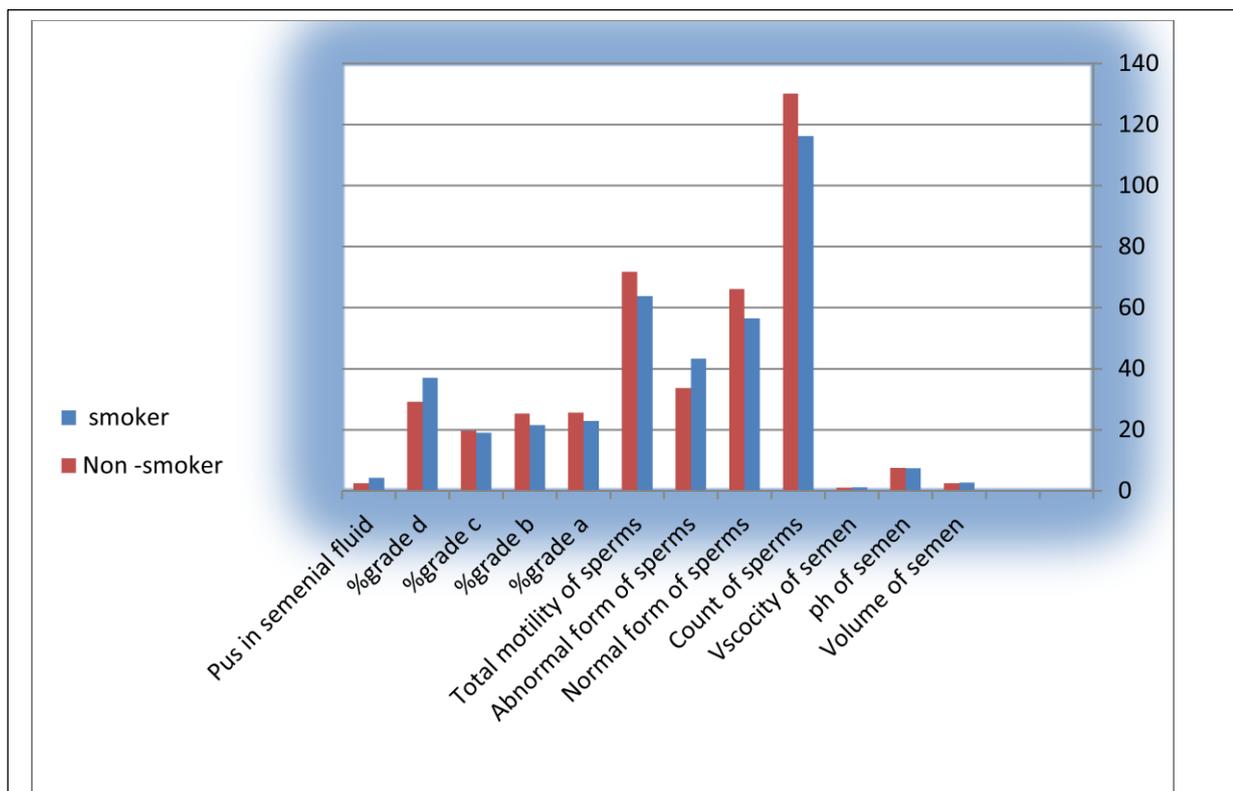


Figure (1): The difference in some seminal fluid parameters between smokers and non-smoker.

Table (3): The difference in seminal fluid variables between smoker and non-smoker.

Result of SFA	Smoker		Non -smoker		P value	
	F	%	F	%		
Normozoospermia	38	38	64	64	0.000	HS
Asthenozoospermia	13	13	11	11	0.000	HS
Oligoasthenozoospermia	11	11	9	9	0.000	HS
Oligoasthenteratosperma	9	9	5	5	0.000	HS
Asthenoteratospermia	14	14	3	3	0.000	HS
Teratizoospermia	9	9	5	5	0.000	HS
Oligozoospermia	6	6	3	3	0.000	HS
Total	100	100	100	100		

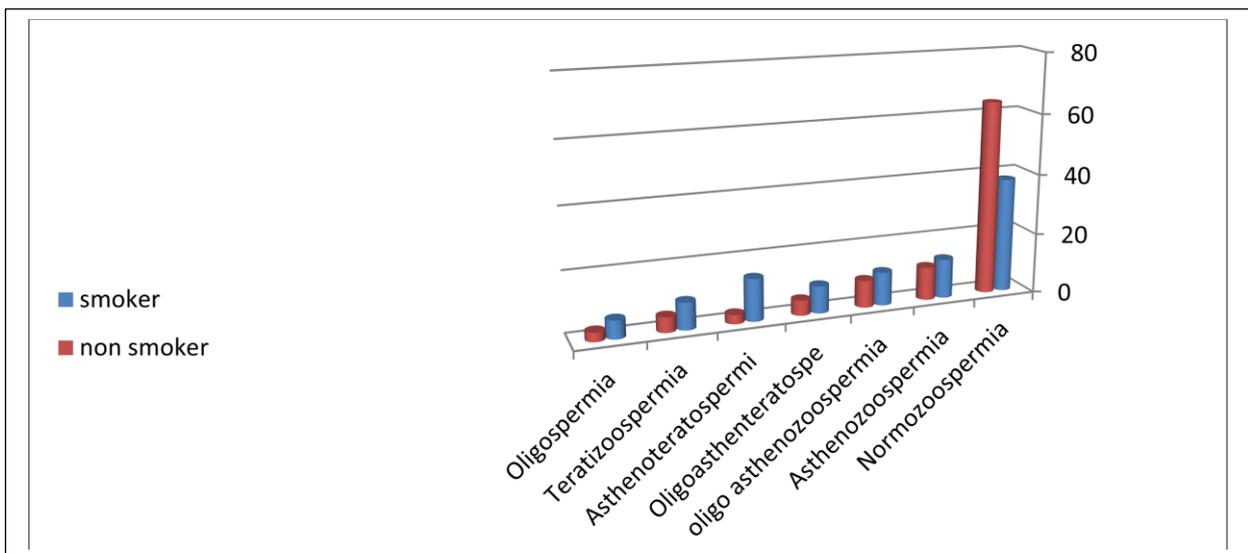


Figure (2): The difference in seminal fluid variables between smoker and non-smoker

Discussion:

The etiology of male infertility in a major proportion of cases is unknown; hence, these situations are provisionally known as idiopathic infertility⁽²⁰⁾. In these cases, smoking may be one of the factors responsible. Smoking is a lifestyle hazard for both active and passive smokers and its effects on fertility status have been less documented. The potential hazardous chemicals present in cigarette smoke may cause sperm abnormality by affecting the chromosomes. About (4%) to (5%) of infertile males showed chromosomal abnormalities in comparison with (0.5% - 0.7%) in the general population⁽²¹⁾.

A consistent number of studies have claimed that cigarette smoking is correlated with alterations in sperm quality such as semen volume, sperm concentration, motility, and morphology⁽²²⁾.

The comparison between pH of both groups (smoker and non-smoker) showed a high difference and this agrees with the result done by Kunzle *et al*⁽⁴⁾.

This study showed that the viscosity of seminal fluid was significantly higher in smoker than non-smoker and this agrees with many researchers who have variously concluded that toxins in cigarette smoker reach the male reproductive system, and their effects, though still under research, are mainly due to their direct interaction with seminal fluid components and the accessory glands, which contribute their secretions to the seminal fluid, leading to its increased viscosity, reduced seminal volume and delayed liquefaction time, thus reducing forward linear progression of spermatozoa, manifesting as Asthnozoospermia⁽⁸⁾.

In this study the normal form of sperms was higher among the non-smoker (66.13) than the smoker (23.583) and this explained by Farkhunda Nadeem *et al.* and Tüzün *et al.*, identified the potential hazardous chemicals present in cigarette smoke which may cause sperm abnormality by affecting the chromosomes^(20,23).

In this study there were a significant difference in mean of sperm count, motility and morphology, the mean count of sperm of smoker (116.186) was lower than the mean in non-smoker which was (130.349) and also the same finding for normal morphology which was lower in smoker (56.43) than the non-smoker (66,13) also the same finding for sperm motility which was higher in non-smoker than the smoker for the mean of total motility which was (63,83) for smoker and (71.76) for non-smoker, the percentage of (grade a) and (grade b) for smoker was lower in the group of smoker than the non-smoker which was (22.86/21,55) for grade a and b respectively for the smoker in comparison with the non-smoker (25.58/25.29) respectively and the result of this study agreed with a meta-analysis study done by (haji *et al*)⁽²⁴⁾ showed 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, also in the study of Lewin⁽²⁵⁾ reported a statistically significant difference in sperm concentration.

In this study Smoking, however, had a statistically non-significant effect on ejaculatory volume ($p > 0.05$) and this agrees with a study who revealed no significant difference in volume of

ejaculation between the smoker and non-smoker⁽²⁶⁾.

The result of this study revealed increased in mean of pus cells (leukocytes) in smoker seminal fluid (4.3 cells) than the non-smoker (2.51 cells) with significant difference (0.01).

The link between cigarette smoking and impaired semen parameters and hence impaired which may be, at least in part, related to the significant increase in leukocyte concentrations in the semen of infertile smokers⁽²⁷⁾.

Because smoking metabolites may induce an inflammatory reaction in the male genital tract with a subsequent release of chemical mediators of inflammation⁽²⁸⁾. This may overwhelm the antioxidant strategies, resulting in oxidative stress (OS) which in turn impair seminal parameters⁽²⁹⁾.

In order to determine the contribution of each of the main semen variables into: Normozoospermia Asthenozoospermia, Oligoasthenozoospermi,

Asthenoteratospermia

Teratizoospermia and Oligozoospermia between smokers as well as non-smokers were distributed according to the presence of individual semen variables or their various combinations observed during semen analysis table (3).

The table (3) revealed that (38%) of the smoker showed Normozoospermia and this was lower than non-smoker which showed that (64%) of samples have Normozoospermia. This finding underscores the fact that smoking certainly has an adverse influence on the semen quality as concluded in several other studies^(4, 8).

This study showed that the dominant seminal fluid variables among the smoker was Asthenoteratospermia (14%) in comparison with the non-

smoker which was only (3%) and Asthenozoospermia was (13%) among the smoker while it was (11%) among the non-smoker and regarding other groups (Oligoasthenozoospermi,

Oligoasthenteratospermia,

Teratizoospermia and Oligozoospermia) was (11%, 9%, 9% and 6%) respectively among the smoker while they were (9%, 5%, 5% and 3%) respectively among the non-smokers, and may be this percentage become different according to type of cigarette.

Asthenoteratospermia was the most dominant semen variable contributing to the semen quality of smokers as well other variables Oligoasthenozoospermi, Oligoasthenteratospermia,

Teratizoospermia and Oligozoospermia was higher among smoker than non-smoker and this revealed that viable and morphologically normal spermatozoa, if they are not actively motile, showing linear forward motion in the seminal fluid, they will fail to fulfill their prime function of traversing the complex route through the female genital tract to seek and fertilize an ovum. In assessing the semen quality of an individual, emphasis has always been on the sperm count and sperm morphology^(30, 4) and smoking contributes to the deterioration of the semen quality of smokers when compared with non-smokers and the direct exposure of spermatozoa to the toxins in cigarettes smoke probably tilts the delicate balance of reactive oxygen species (ROS) that are produced by spermatozoa for their special functions like decapitation⁽³⁰⁾.

Increased quantities of ROS have been shown to be detrimental to the DNA of spermatozoa, thus producing a negative effect on the viability and morphology of spermatozoa⁽³¹⁾. Thus, smoking plays a role in producing Asthenozoospermia

in otherwise normal and viable spermatozoa, and can be a very subtle “early indicator” of deterioration in semen quality⁽³²⁾.

Conclusions:

The smoking has bad effect on seminal fluid parameters and there was a difference between the two groups and the smoking can affect and reduce the fertility of the male.

Smoking can be a cause of infertility in males especially those with idiopathic infertility.

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