The Study of Detrimental Outcomes of Cigarette Smoking and Alcohol Intake on Semen Quality Parameters and some Reproductive Hormone profile (LH, FSH and Testosterone) in Infertile Patients

Basim Khamees Gootee

Thi-Qar University-College of Science-Department of Biology, Iraq

Applied Embryology Subspecialty

Abstract

This study was undertaken to evaluate the injurious achieve of cigarette smoking and alcohol consumption on semen parameters and reproductive hormones profile (LH, FSH and Testosterone) on infertile patients affected with male infertility factors. Forty-five infertile patients were subjected into three groups (15 patients with mild smoking, 15 patients with moderate smoking, and 15 patients with heavy smoking). semen samples were collected by masturbation and prepared by direct layering technique. The questionnaire included the numbers of cigarette per day and the duration of smoking counted with years and the smoking status of the female partners.

The sperm function test and reproductive hormonal analysis were analyzed and semen prepared by standard semen parameters. (sperm concentration, sperm motility, progressive sperm motility, sperm agglutination, and sperm morphology) were evaluated. Smoking was classified as mild smokers (≤ 10 cigarette per day), moderate smokers (> 10 cigarette and ≤ 10 cigarette per day) and heavy smokers (> 10 cigarette per day). The results indicated a highly significant ($P<0.001$) differences in sperm functions parameters for all groups of smoker men in *in vitro* post-preparation compared with pre-preparation technique. The reproductive hormones LH, FSH and Testosterone concentration were significantly ($P<0.001$) lower in smoker men in post-preparation compared with pre-preparation. It was concluded that smoking and alcohol drinking was positively correlated to decreased semen quality and decreased semen reproductive hormonal profile.

1- Introduction

There are strong evidences indicate that the smoking behavior is related to community factors, particularly the influence of parents and peer groups (1). Taste and smell also influence the inclination to smoke where exciting sensory organs in the lips, mouth and throat provide sensations of touch, taste and irritation (2). Also, it has been suggested that high negative mood variability is a risk factor for future smoking escalation and that its mood-stabilizing effects may reinforce and maintain daily cigarette use among youths (3). However, with much debate for its impact on various semen parameters, it is regarded as an infertility risk factor (4). Cigarette smoke consists of gases, vaporized liquids and particles, many of which are minute droplets. About 4000 compounds are generated by a lit cigarette through variety of processes including; hydrogenation, pyrolysis, oxidation, decarboxylation and dehydration (5). Smoke is separated into two phases; gaseous and particulate phases. The major constituents that affect health are: nicotine, tar in the particulate phase and carbon monoxide in the gaseous phase (6). Main stream smoke emerges into the environment after it is drawn through the cigarette, filtered by the smoker's own lungs and then exhaled. However, elevation stream smoke arises from burning end of the cigarette and enters directly into environment (7). Passive smoking refers to the involuntary inhalation of tobacco smoke present in the air breathed (8).

In males, it has been suggested that cigarette smoking negatively affects every system involved in reproductive process. Spermatozoa from smokers have reduced fertilizing capacity, and embryos display

lower implantation rates (9). There is a negative impact of the cigarette smoking on human semen parameters correlated with cigarettes smoked/day and the smoking duration (10). Also, most authors indicated argued that smokers demonstrate lower semen volume, sperm count, sperm motility and viability compared with non-smokers. In addition, smokers showed increased seminal leukocytes, oval sperm percentage, head-piece spermatozoa defects percentage and spermatozoa with cytoplasmic droplets, severe DNA damage, which might prevent oocyte fertilization or the development of the embryo (11). Moreover, chromosome damage was observed in Golgi-phase or cap-phase spermatids, showing frequencies of high levels in infertile smokers and low levels in infertile non-smokers (12). Nicotine has a significant influence on sperm count and morphology and the seminal nicotine and cotinine levels correlated with degree of reported exposure (13). Nicotine can alter hypothalamic-pituitary axis through stimulation of growth hormone, cortisol, and vasopressin and oxytocin release, which in turn inhibit LH and PRL release (14). The mean 17 beta-estradiol (E) level was higher and mean levels of LH, FSH, T and PRL were lower in smokers compared with non-smokers, whereas mean levels of T and dehydroepiandrosterone (DHEA) did not differ (15). However, other studies indicated that increased free and total serum T and decreased PRL in smokers and was significantly associated with a decrease in seminal PRL and observed a positive dose-response relation between smoking and T, LH and LH/free T ratios (16,17).

2. Subjects, Materials and Methods

2.1. Subjects

Forty-five infertile patients (15 patients with mild, 15 patients with moderate, and 15 patients with heavy smoking) were selected from Al-Hussein Teaching Hospital/ Thi-Qar Health directorate/laboratory section. The selection of infertile patients was based on physical examination and assessment was by using questionnaire including numbers of cigarette per day and duration of smoking counted with years and smoking index. Each infertile patient was to have baseline semen samples including the parameters of sperm function test and reproductive hormone analysis.

2.2. Semen preparation technique (DLT)

The semen was prepared by using a direct layering technique. However, 1ml of prepared IVF culture medium (Medi-Cult Company, Denmark) was added to the test tube, and then 1ml of the liquefied semen was layered beneath a culture medium. After incubation for 30 minute in 5% CO_2 at 37ºC, 10µl. of mixture was aspirated by pasture pipette and examined under light microscope at 400X magnification for assessment parameters of sperm function.

2.3. Clinical and laboratory investigation:

2.3.1. Seminal fluid collection and analysis:

Semen samples were collected by masturbation after 3-5 days abstinence into a dry, clean, and sterile Petri-dish labeled with name and age of patient, period of abstinence and time of collection. The specimens were placed in the incubator at 37ºC to allow the semen liquefaction. WHO criteria for normal semen values were applied (WHO, 2010).

However, smoking index (SI) is cigarette smoking number/day×smoking years; mild (<200SI), moderate (200-600SI), and heavy (>200SI). For preparation technique, sperm prepared and incubated for 30 minute in 5% CO_2 at 37ºC after in vitro sperm processing.

2.3.2. Blood sampling and hormonal assessment

The blood samples (3-5 ml fresh blood) was drawn and collected in a clean, disposable plastic tube from anterior cubital vein under aseptic condition for hormonal analysis. Serum concentrations of FSH, LH and

T were assessed using MiniVIDAS apparatus (VIDAS 12, 1992, Biomerieux Company, France) through an enzyme linked fluorescent assay (ELFA) technique.

3. Statistical analysis

Statistical analysis was performed with the SPSS version 12.00 by the Statistical Package for Social Sciences software to compare difference between pairs of groups. P-value < 0.05 was used as a level of statistically significance.

4. Results

4.1. Parameters of SFA and in vitro sperm processing

According to cigarette smoking and alcohol intake, infertile patients were classified into three groups as smokers with mild, moderate, and heavy smoking are shown in table (1). Results of SFA parameters for those subjects are shown in table (2). It was observed that parameters of SFA for heavy smokers were more deviated from normal parameters of SFA as compared with mild and moderate smokers. Table (3) shows the results of in vitro sperm preparation for smokers and smoker's subjects undergoing sperm processing. However, the sperm concentration and percentage of sperm agglutination were reduced significantly (P<0.001) post in vitro sperm preparation when compared with pre-activation. In contrast, the percentage of sperm motility, progressive sperm motility, and normal sperm morphology were highly significant (P<0.001) increased post activation in vitro as compared to pre-activation of human spermatozoa.

4.2. Hormonal analysis concentration

According to cigarette smoking and alcohol intake, three reproductive hormone concentrations FSH, LH, and testosterone were assessed in this study. It was observed that FSH, LH, and testosterone (T) were more deviated from normal value in all smokers men especially in heavy smokers as compared with mild and moderate smokers (table 4 and figure 4; respectively). However, low levels of FSH, LH, and testosterone in all smokers subjects and reduced significantly (P<0.001) post in vitro sperm preparation when compared with pre-activation.

Table (1): Frequency and distribution of standardization cigarette smoking status and alcohol intake including in this study

Smoking status	Standardization of cigarette smoking and alcohol intake			
	Number of cigarette	Number of years	Smoking index	Standard indicator
Mild smoking and alcohol intake 1/2 bottle daily	1-35	22	770	≤ 10 cigarette per day<200SI
Moderate smoking alcohol intake1.5 bottle daily	36-60	24	864	> 10 cigarette per day 200-600SI
Heavy smoking alcohol intake2 bottle daily	≥60	27	1620	> 10 cigarette per day >200SI

Values are Mean ± S.E.M

Total No. of infertile patients=45

Mean of age for infertile patients (31.35 ± 0.66 years)

Mean of duration of infertility for infertile patients (5.66 ± 0.33 years)

Mild (<200SI), moderate (200-600SI), and heavy (>200SI)

Mild smokers (≤ 10 cigarette per day

Moderate smokers (> 10 cigarette and ≤ 10 cigarette per day)

Heavy smokers (> 10 cigarette per day)

Table (2): Seminal fluid analysis parameters of infertile men distributed according to smoking status.

Parameters	Mild Smoking	Moderate Smoking	Heavy smoking
Sperm concentration ($\times 10^6$ sperm/ml)	38.65 ± 3.23a	26.42 ± 2.20b	22.32 ± 2.18c
Sperm Motility (%)	52.36 ±1.40a	43.28 ±1.32a	38.20 ±1.40a
Progressive Sperm motility (%)	30.22 ±1.00a	26.20 ±1.14b	22.20 ±1.12c
Sperm agglutination (%)	15.36 ±1.07a	12.28 ±1.03b	10.20 ±1.00c
Normal sperm morphology (%)	50.54±1.65a	42.54±1.54b	38.54±1.42c

Values are Mean ± S.E.M

Total No. of infertile patients=45

Mean of age for infertile patients (31.35 ± 0.66 years)

Mean of duration of infertility for infertile patients (5.66 ± 0.33 years)

Smoking index (SI) is a number of cigarette smoking /day×smoking years

Mild (<200SI), moderate (200-600SI), and heavy (>200SI)

Mild smokers (≤ 10 cigarette per day)

Moderate smokers (> 10 cigarette and ≤ 10 cigarette per day)

Heavy smokers (> 10 cigarette per day)

Table (3): Sperm preparation technique by simple layering methods of infertile men distributed according to smoking status

Parameters	Mild Smoking n=15		Moderate Smoking n=15		Heavy Smoking n=15	
	Pre-preparation	Post-preparation	Pre-preparation	Post-preparation	Pre-preparation	Post-preparation
Sperm concentration ($\times 10^6$ sperm/ml)	38.65 ± 3.23	26.60 ± 3.20 a	36.42 ± 2.20	22.33 ± 2.12 b	22.32 ± 2.18	18.21 ± 2.12 c
Sperm Motility (%)	52.36 ± 1.40	68.22 ± 0.40 a	43.28 ± 1.32	55.20 ± 1.02 b	38.20 ± 1.40	56.17 ± 1.23 c
Progressive Sperm motility (%)	30.22 ± 1.00	42.12 ± 1.13 a	26.20 ± 1.14	32.25 ± 1.27 b	22.20 ± 1.12	30.20 ± 1.10 c
Sperm agglutination (%)	15.36 ± 1.07	05.27 ± 1.15 a	12.28 ± 1.03	03.12 ± 1.00 b	10.20 ± 1.00	02.10 ± 1.13 c
Normal sperm morphology (%)	50.54± 1.65	58.52± 1.53 a	42.54± 1.54	56.32± 1.26 b	38.54± 1.42	51.40± 1.20 c

Values are Mean ± S.E.M

Total No. of infertile patients=45

Mean of age for infertile patients (31.35 ± 0.66 years)

Mean of duration of infertility for infertile patients (5.66 ± 0.33 years)

a: means a highly significant (P< 0.001) difference from pre-activation

Smoking index (SI) is a number of cigarette smoking /day×smoking years

Mild (<200SI), moderate (200-600SI), and heavy (>200SI)

Mild smokers (≤ 10 cigarette per day)

Moderate smokers (> 10 cigarette and ≤ 10 cigarette per day)

Heavy smokers (> 10 cigarette per day)

Table (4): Reproductive hormone concentration for serum (FSH, LH, and Testosterone) in infertile smokers men distributed according to smoking status and alcohol intake.

Infertile smokers	Numbers	Hormonal levels Mean ± S.E.M		
		FSH (mIU/ml)	LH (mIU/ml)	T (ng/ml)
Mild smoking and alcohol intake n=15	15	7.34 ± 4.29a	6.33 ± 2.53a	4.02 ± 1.62a
Moderate smoking and alcohol intake n=15	15	08.50 ± 5.46b	12.96 ± 9.36b	06.23±1.08b
Heavy smoking and alcohol intake n=15	15	06.42±1.03c	11.64±2.82c	04.53± 1.13c

Values are Mean ± S.E.M

Normal levels of T = 3.0 -10.6 ng/ml, FSH= 1.70 -12.0 mIU/ml; LH = 1.1-7.0 mIU/ml

Total No. of infertile patients=45

Mean of age for infertile patients (31.35 ± 0.66 years)

Mean of duration of infertility for infertile patients (5.66 ± 0.33 years)

Smoking index (SI) is a number of cigarette smoking /day×smoking years

Mild (<200SI), moderate (200-600SI), and heavy (>200SI)

Mild smokers (≤ 10 cigarette per day)

Moderate smokers (> 10 cigarette and ≤ 10 cigarette per day)

Heavy smokers (> 10 cigarette per day)

Figure (1): Demonstration of Seminal fluid analysis parameters of infertile men distributed according to standardization smoking status.

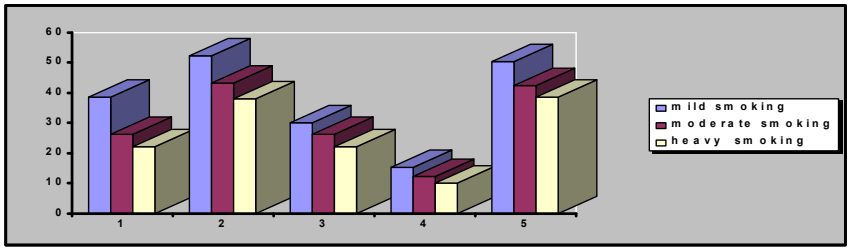


Figure (2): in vitro sperm preparation technique of semen parameters for mild cigarette smoking of infertile men in this study.

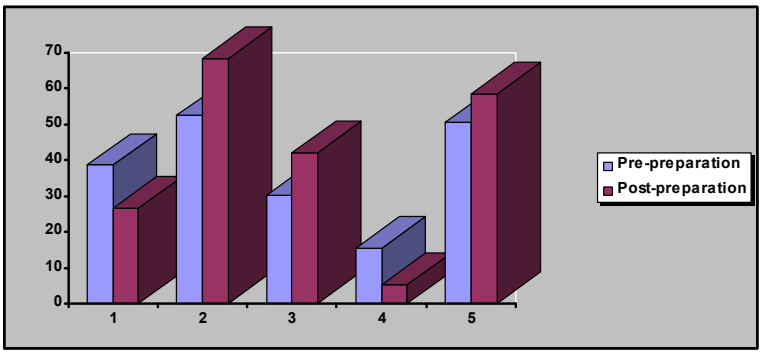


Figure (3): In vitro sperm preparation technique of semen parameters for moderate cigarette smoking of infertile men in this study.

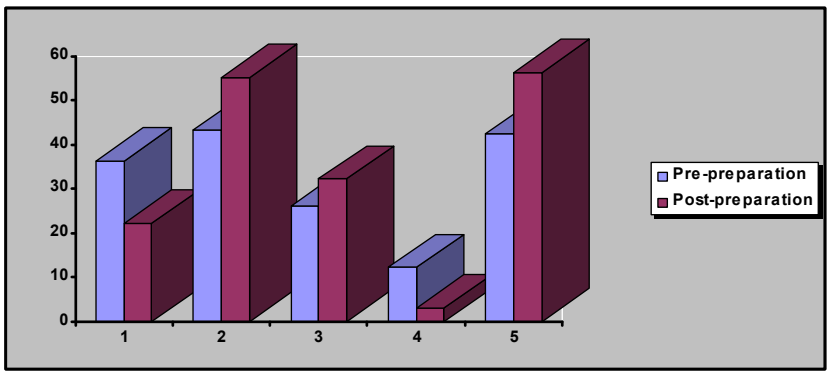


Figure (4): In vitronique of semen parameters for heavy cigarette smoking of infertile men in this study.

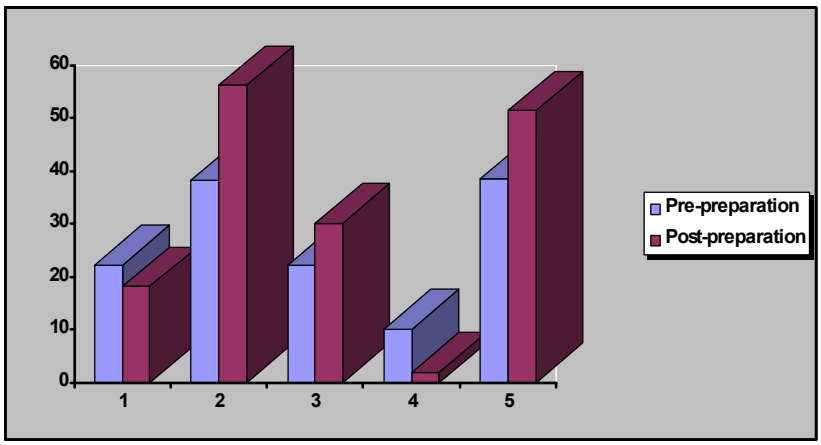
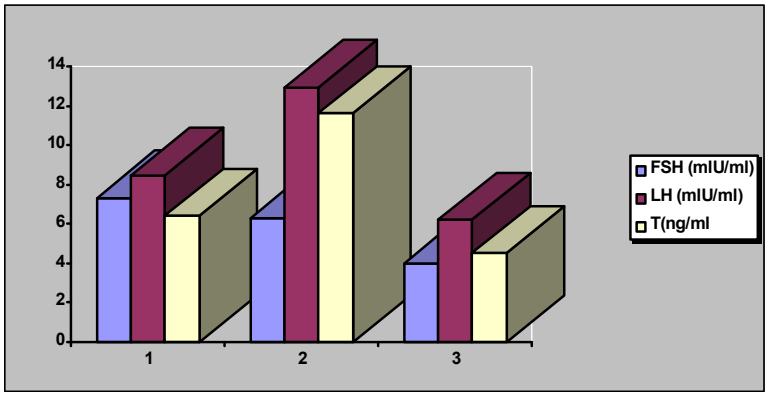


Table (5): Demonstration of hormone concentration for serum (FSH, LH, and T) in infertile smokers men distributed according to smoking

status and alcohol intake.



4-Discussion

Smoking is a lifestyle hazard for both active and passive smokers. The cigarette smoking has various detrimental effects on male reproductive system specifically on semen parameters (18). However, it was mentioned that the cigarette smoking adversely effects on human Leydig cell metabolism and function (19). In addition, the influence of smoking on the ability of men to have children may be due to impaired spermatogenesis secondary to various hormonal alterations and DNA damage (20).

It was reported that cigarette contains large numbers of substances, including nicotine, carbon monoxide, carcinogens, mutagens, cyanogens, and aneugens such as radioactive polonium (21). Furthermore, the inhalation of cigarette substances through active or passive smoking leads to absorption through the pulmonary vasculature and blood-borne circulation throughout the body (22). Additionally, it was assessed that cigarette substances could end up in the seminal plasma of smokers via various modes of diffusion and active transport (23). Also, chemical agents or mutagens may adversely affect hormonal control of spermatogenesis or may directly affect Sertoli cells within the semineferous tubules in smokers with disturbance of plasma membrane phospholipids asymmetry on sperm surface (24).

The highly significant correlation between ability of male to achieve pregnancy and cigarette smoking. Furthermore, it was mentioned that smoking affect directly on the ability of seminal plasma to maintain sperm viability and longevity by measuring the direct effect of seminal plasma obtained from smokers and non smokers on standard parameters of semen analysis (25). However, the direct effect on male germ cells are plausible for both biologic and toxicologic reasons and also indicated that smoking was associated with detrimental effects on sperm concentration, motility, and morphology with increase in disomic sperm and decrease in specific aspects of semen quality (26). As well as, it was noticed that seminal plasma from non smokers contains a protective substance or factor involved in protection of sperm against cigarette smoke metabolites and this substance may be decreased or inactivated in seminal plasma of smokers (27).

The long-term effects of chronic alcohol intake include erectile dysfunction, reduced libido, and gynecomastia. One mechanism of these effects is a reduction in serum testosterone concentration caused by decreased testicular production and increased metabolic clearance in liver (28). It is thought that alcoholism and hepatic cirrhosis cause alterations in the HPG axis, resulting in testicular dysfunction. In addition, oxidation of alcohol competes with testicular production of testosterone (29). These mechanisms lead to subsequent decrease in semen volume and sperm density. Another factor appears to be an elevation in serum

estrogencaused by peripheral conversion of testosterone to estrogen through increased activity of enzyme aromatase, which is present both in the liver and in peripheral fat cells (30). The light alcohol ingestion does not appear to interfere with semen quality. However, excessive acute alcohol intake does have adverse effects on male fertility by causing decreased serum testosterone concentrations (31). Moreover, impairment of spinal reflexes, also caused by excessive alcohol abuse, leads to reduced sensation and innervations of the penis, and thus may also contribute to erectile dysfunction (32).

The smoking cigarette has a significant negative impact on sperm production, motility, and morphology. However, several reports demonstrated that mutagenic and carcinogenic components of cigarette smoke have adverse effects on rapidly dividing cells, including germ cells in testis (33). However, recently observed that differences were seen in testicular volume, FSH, LH, PRL and testosterone levels, or sperm concentration, motility, and morphology in a population of fertile patients who smoke or drink coffee compared to patients that do not have these habits (34). Estradiol impairs spermatogenesis via several different mechanisms, including alteration of the HPG axis. Studies have also show that elevated E_2 levels can cause increased catecholamine levels, which in turn can produce ischemia of the seminiferous tubules (35).

5. Conclusion: It was concluded that a strong positive correlation between smokers and alcohol drinking side direction and decline semen quality and reproductive hormones from on the part of infertile patients.

6. Recommendation: Further studies are recommended to assess the detrimental effect of cigarette smoking on DNA damage and embryo quality after in vitro fertilization and embryo transfers (IVF-ET).

References

1- Antoaneta B., Maria IK., Petia T. Mihail M. (2004): Effect of cigarette smoking on sperm plasma membrane integrity and DNA fragmentation. Inter. J. Androl.5:296.

2- Aydos K., Guven MC., Can B. and Ergun A. (2001): Nicotine toxicity to the ultrastructure of the testis in rats. B.J.U. Int. 88:622-6.

3- Benowitz NL. (1997): Systemic absorption and effects of nicotine from smokeless tobacco. Adv. Dent. Res. 11:336-41.

4- Bersinger NA. (2003): Semen quality of male smokers and non smokers in infertile couples. Fertil. Steril. 79:287-91.

5- Kunzle R., Mueller MD., Hanggi W., Birkhauser MH., Drescher H. and Bersinger NA. (2003): Semen quality of male smokers and non smokers in infertile couples. Fertil. Steril. 79:287-91.

6- Lando HA., Haddock CK., Klesges RC., Talcott GW. and Jensen J. (2002): Smokeless tobacco use in a population of young adults. Am. J. Public. Health. 92:250-6.

7- Rodriguez-Rigau LJ., Smith KD. and Steinberger E. (1982): Cigarette smoking and semen quality. Fertil. Steril. 38:115-6.

8- Saleh RA., Agarwal A., Sharma RK., Nelson DR. and Thomas AJ Jr. (2002): Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil. Steril. 78:491-9.

9- Stillman JR. (1986): Smoking and reproduction. Fertil. Steril. 46:545-66.

Vine MF., Tse CK., Hu P. and Truong KY. (1996): Cigarette smoking and semen quality. Fertil. Steril. 65:835-42.

10- World Health Organization (WHO) (1999): Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 4th ed. Cambridge, Cambridge University Press UK. Pp.8-11.

11- Zavos PM. (1989): Cigarette smoking and human reproduction: effects on female and male fecundity. Fertil. Steril. 12:35– 46.

12- Zavos PM., Kaskar K., Correa JR., Aslanis P. and Koundouros S. (2005): The impact of cigarette smoking on male and female sexual behavior:Who sets the pace. Asian. J. Androl. 69: 430-444.

13- Zhao GQ. and Garbers DL. (2002): Male germ cell specification and differentiation. Dev. Cell. 2: 537-547.

14- Brugo-Olmedo S., Chillik C. and Kopelman S. (2001): Definition and causes of infertility. Reprod. Biomed. Online. 2:41-53.

15- Zhang JP., Meng QY. and Wang Q. (2000): Effect of smoking on semen quality of infertile men in Shandong, China. Asian J. Androl. 2:143-6.

16- Kulikauskas V., Blaustein D. and Ablin RJ. (1985): Cigarette smoking and its possible effects on sperm. Fertil. Steril. 44:526-8.

17- Rantala ML. and Koskimies AI. (1987): Semen quality of infertile couple's comparison between smokers and non-smokers. Andrologia. 19:42-6.

18- Holzki G., Gall H. and Hermann J. (1991): Cigarette smoking and sperm quality. Andrologia. 23:141-4.

19- Chia SE., Ong CN. and Tsakok FM. (1994): Effects of cigarette smoking on human semen quality. Arch. Androl. 33:163-8.

20- Kunzle R., Mueller MD. and Hanggi W. (2003): Semen quality of male smokers and nonsmokers in infertile couples. Fertil. Steril. 79:287-91.

21- Zinaman MJ., Brown CC., Selevan SG. and Clegg ED. (2000): Semen quality and human fertility: a prospective study with healthy couples. J. Androl. 21:145-53.

22- Saaranen M., Suonio S., Kauhanen O. and Saarikoski S. (1987): Cigarette smoking and semen quality in men of reproductive age. Andrologia. 19:670-6.

23- Pasqualotto FF., Sobreiro BO., Hallak J., Pasqualotto EB. and Lucon AM.(2006): Cigarette smoking is related to a decrease in semen volume in a population of fertile men. B.J.U. Int. 97:324-6.

24- Arabi M. and Moshtaghi H. (2005): Influence of cigarette smoking on spermatozoa via seminal plasma. Andrologia. 37(4):119-24.

25- Zavos PM., Correa JR., Antypas S., Zarmakoupis Zavos PN. and Zarmakoupis CN.(1998): Effects of seminal plasma from cigarette smokers on sperm viability and longevity. Fertil. Steril. 69(3):425-9.

26- Mehran A. (2005): The toxic effect of seminal plasma from smokers on sperm function in non-smokers. Zhonghua. Nan. Ke. Xue. 11(9):647-51.

27- Baird DD. and Wilcox AJ. (1986): Future fertility after prenatal exposure to cigarette smoke. Fertil. Contracept. 46(3):368-72.

28-] Ratcliffe JM., Gladen BC.,Wilcox AJ. and Herbst AL.(1992): Does early exposure to maternal smoking affect future fertility in adult males. Reprod. Toxicol. 6(4):297-307.

29- Jensen MS., Mabeck LM., Toft G., Thulstrup AM. and Bonde JP. (2005): Lower sperm counts following prenatal tobacco exposure. Hum. Reprod. 20(9):2559-66.

30- Joesbury KA., Edirisinghe WR., Phillips MR and Yovich JL. (1998): Evidence that male smoking affects the likelihood of a pregnancy following IVF treatment: application of the modified cumulative embryo score. Hum. Reprod. 13(6):1506-3.

31- MacMahon B., Trichopoulous D., and Cole P. and Brown J. (1982): Cigarette smoking and urinary estrogens. N. Engl. J. Med. 307: 1062.

32- Stillman JR., Rosenberg HJ. and Sachs PB. (1986): Smoking and reproduction. Fertil. Steril. 46: 545.

33- Blake CA., Norman RL., Scaramuzzi RJ. and Sawyer CA. (1973): Inhibition of the proestrous surge or prolactin in rat by nicotine. Endocrinol. 92: 1334.

34- American Thoracic Society. (1996): Cigarette smoking and health. Am. J. Respir. Crit. Care Med. 153: 861-865.

35- Evans JH., Fletcher J., Torrance M. and Hargreave TB. (1981): Sperm abnormalities and cigarette smoking. Lancet 1:627: 1981

Heavy smokers (> 10 cigarette per day)

دراسة التأثير الضار للتدخين وتناول الكحول على متغيرات السائل المنوي ومستوى تركيز بعض الهرمونات التكاثرية لمرضى العقم في محافظة ذي قار

الخلاصة: تهدف هذه الدراسة الى تقييم التاثير الضار لتدخين السكائر وشرب الكحول على متغيرات السائل المنوي ومستوى الهرمونات التكاثرية لمرضى العقم المصابين بعوامل متعددة للعقم الذكري في محافظة ذي قار. تم اختيار المرضى وعددهم خمسة واربعون مريضاً ومقسمين بواقع ثلاث مجاميع متساوية العدد وجميعهم مدخنين وشاربي كحول (15ذات تدخين خفيف،15ذات تدخين متوسط،،15ذات تدخين عالي) وعينات السائل المنوي تم جمعها بطريقة الاستمناء بواسطة غرفة خاصة لجمع المني وتم فحصها بطريقة التقنية الطباقية المباشرة للنطف.

ان فحوصات كفاءة النطف ومستوى تركيز الهرمونات التكاثرية تم تقييمها اعتماداً على المستوى القياسي المعتمد عالمياً لمرضى العقم. ان اعتماد اختبارات كفاءة النطف والمتضمن تركيز النطف، حركة النطف، الحركة التقدمية للنطف، تلازن النطف، والنسبة المئوية للنطف السوية حسب مقررات منظمة الصحة العالمية. ان السائل المنوي تم تحضيره في ظروف قاسية (5% CO_2 at 37ºC) بعد اجراء عملية التنشيط خارج الجسم. ان مؤشر او دلالة التدخين والمسمى (Smoking Index:SI) تم اعتماده في هذه الدراسة والذي هو عبارة عن عدد السكائر المدخنة في كل يوم مضروباً في عدد سنوات التدخين.

اظهرت نتائج هذه الدراسة وجود فرقاً معنوياً عالياً في فحوصات كفاءة النطف لجميع المرضى المدخنين بعد اجراء عملية تحضير النطف خارج الجسم مقارنة بالنتائج قبل تحضير النطف. اضافة الى ذلك وجود فرق معنوي عالي لمستوى الهرمونات التكاثرية (الهرمون المحفز للجريبات المبيضيةFSH والهرمون المصفرLH والهرمون الخصويTestosterone) بقلة مستويات هذه الهرمونات في مجاميع المرضى المدخنين بعد اجراء تنشيط النطف مقارنة بنتائج قبل اجراء تقنيات التنشيط. نستنتج من خلال هذه الدراسة هناك علاقة عالية وقوية بين تدخين السكائر وشرب الكحول من جهة وقلة وردائة متغيرات تحليل النطف والهرمونات التناسلية للمرضى المدخنين من جهة اخرى. هنالك توصية باجراء دراسة لمعرفة مدى التاثير الضار لتدخين السكائر وشرب الكحول على ضرر الحامض النووي ونوعية الجنين بعد اجراء تقنيات الاخصاب الخارجي ونقل الاجنة (اطفال الانابيب باستخدام تقنية الاكزي).