

The detrimental effect of cigarette smoking on semen parameters and sperm plasma membrane integrity in infertile patients undergoing intra-uterine insemination

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Abstract:

The present study was designed to assess the effect of cigarette smoking on semen parameters, hypo-osmotic swelling (HOS) test (%) and outcome of intra-uterine insemination (IUI). In this study, one hundred infertile males were involved and according to cigarette smoking were divided into 55 smokers and 45 non smoker infertile couples. From each male, semen samples were collected and the sperm parameters including sperm concentration, sperm motility, progressive sperm motility, normal sperm morphology, and HOS-test were evaluated according to standard World Health Organization (WHO) criteria. For IUI, the sperm prepared using direct swim-up technique through incubation for 30 minute in 5% CO₂ at 37°C. The results of the present study demonstrated that the sperm parameters, HOS-test (%) and IUI outcomes for non smoker infertile males was higher than smokers. In addition, the results of sperm parameters and HOS-test (%) for smokers were more deviated from normal range of criteria of WHO than non smokers. Non significant differences ($P>0.05$) in the sperm HOS-test were assessed between non smokers and smokers.

From the results of the present study, it was concluded that the cigarette smoking have several impacts on sperm functions and integrity of plasma membrane, as well as sperm fertilizing ability. Further studies are recommended to assess the effect of cigarette smoking on DNA damage and embryo quality after IVF-ET.

Introduction

The cigarette smoke contains a large number of substances including nicotine, carbon monoxide, carcinogens, and mutagens such as radioactive polonium (1). However, inhalation of cigarette smoke through active or passive smoking leads to absorption of these substances through pulmonary vasculature and blood-borne circulation throughout the body. It was become important to assess the side effects of cigarette smoking on male reproduction performance especially on fertilization potential for use in various assisted reproductive technology (2).

A number of researchers have mentioned various detrimental effects of cigarette smoking on sperm parameters and hypo-osmotic swelling test (3). 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 (4). The influence of smoking on fertilizing ability of men may be caused by impaired spermatogenesis secondary to various hormonal alterations (5). It is also possible that those same substances could end up in the seminal plasma of smokers via various modes of diffusion and active transport (6).

In general, HOS-test is easy to score and give additional information on the functional integrity of sperm plasma membrane (7). Moreover, the functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction, and binding of spermatozoa to the egg surface (8). The hypo-osmotic swelling (HOS) test introduced as a clinical, physiological, and non deleterious test by Jeyendran *et al.* (9). However, WHO considered HOS-test as an optional, additional, and viability test (7).

Jeyendran *et al.* (10) confirmed a good correlation between human sperm HOS-test and other sperm function tests (SFTs), sperm penetration assay (SPA), and IVF outcome. The functional damage to the sperm plasma membrane as measured by low HOS test has been associated with very poor pregnancy rate (PRs), implantation failure, not failed fertilization (11). WHO (7) considered HOS test normal for a semen sample if more than 50% of spermatozoa undergo tail swelling and associated with normal fertilization rates, while abnormal if less than 50% show tail swelling and had poor fertilization rates (12). Therefore, this study was designed to assess the effect of cigarette smoking on semen parameters, hypo-osmotic swelling (HOS) test score and outcome of intra-uterine insemination (IUI).

2. Subjects, Materials and Methods

2.1. Subjects:

In this study, one hundred infertile couples (55% smokers vs. 45% non smokers) were enrolled and semen samples were obtained at Institute of Embryo Research and Infertility Treatment/Al-Nahrain University. The semen samples were collected by masturbation after 3-5 days abstinence and allow liquefying at 37 °C in 5% CO₂ incubator for 30 minutes. The liquefied semen is then carefully mixed for few seconds, and then the specimen was assessed by macroscopic and microscopic examinations including sperm concentration, sperm motility, progressive sperm motility, normal sperm morphology, and HOS-test, WHO criteria for normal semen values were applied as mentioned in details by Kouty (13).

2.2. Hypo-osmotic swelling test

The HOS test was performed after examination of standard semen parameters by mixing 0.1 mL of semen with 1.0 mL of a 150 mOsm/ kg NaCl as a hypo-osmotic solution. The mixture was incubated for 30 minute at 37 °C in 5% CO₂ incubator. Then, 10 µL of the mixture was placed on a slide and mounted with a cover and examined immediately at a magnification of 40X objective under a light microscope. A total of 100 spermatozoa were counted in at least ten different fields. The percentage of HOS-reacted spermatozoa (with coiled and swollen tail) and non-reacted spermatozoa (with straight or non swollen tails) were calculated. Of one hundred spermatozoa were examined, at least 50% of swollen spermatozoa were considered normal (7,9).

2.3. Semen preparation for intrauterine insemination (IUI)

The sperm was prepared for IUI using a direct Swim-up technique. However, 1 mL of prepared IVF culture medium (Medi-Cult Company, Denmark) was added to the test tube, and then 1 mL of liquefied semen was layered beneath a culture medium. After incubation for 30 minute in 5% CO₂ incubator at 37°C, 10µL from upper culture medium was aspirated by pasture pipette and examined under light microscope at 400X magnification for assessment sperm parameters and HOS-test (13).

2.4. Timing of IUI and ovarian stimulation protocol

The vaginal ultrasounographic examinations for follicular size were starting throughout follicular phase of menstrual cycle. Females were given clomiphene citrate (50 mg) two times daily for 5 days (cycle day; 2-6 day), then recombinant FSH (Gonal-F; 75 IU; Serono; Italy) for another 5 days (cycle day; 7-11). At least, when one ovarian follicle reaches ≥18 mm average diameters associated with a serum E₂ of at least 200pg/mL, Human chorionic gonadotropin (hCG; 10000IU; Serono; Italy) was injected, and later IUI was done after 36 hours. IUI was performed by threading a very thin flexible rubber catheter through the cervix and injected prepared sperm into the uterine cavity.

2.5. Statistical analysis

Statistical analysis was performed with the SPSS (Statistical Package for Social Sciences Software; version 12). The data analysis was done using paired sample t-test to assess statistical differences in the results of SFTs and sperm HOS test. Mean and standard error of

mean (S.E.M) obtained from crude data to compare between seminal parameters. P-value < 0.05 was used as a level of statistically significant.

3. Results

The results of SFA for smoker and non smoker patients are shown in the table (1). It was observed that the parameters of SFA for smokers were more deviated from normal WHO criteria of SFA as compared to non smokers. However, semen volume, liquefaction time and pH were within normal criteria for both groups of infertile patients. For same groups, also, sperm concentration, motility (%) and normal morphology (%) were observed within normal criteria. In contrast, semen viscosity, sperm progressive motility (%), sperm agglutination (%) and sperm HOS test (%) were noticed within abnormal means (Table 1).

In the non-smoker subjects, sperm HOS test have positive and significant correlations with percentages of sperm motility ($r= 0.732$; $P= 0.001$), progressive sperm motility ($r= 0.463$; $P= 0.001$), and normal sperm morphology ($r= 0.321$; $P= 0.03$). Similarly, positive and significant correlations were observed between HOS test and sperm motility ($r= 0.571$; $P= 0.001$), progressive sperm motility ($r= 0.274$; $P= 0.04$), and normal sperm morphology ($r= 0.294$; $P= 0.02$) for smoker infertile patients.

The number of clinical pregnancies was 18 % after *in vitro* sperm activation and IUI technique. There was a significant reduction ($P<0.05$) in the number of clinical pregnancies for infertile couples where male partners is smokers (No. =6) as compared to non smokers (No. =12) as shown in figure (1). It was worthwhile, in this study; all 18 clinical pregnancies were achieved for infertile couples where their male have normal percentages of sperm HOS test scores as presented in the figure (2). Therefore, no male partner has abnormal percentage of sperm HOS test of 100 infertile couples where his wife able to be pregnant. In contrast to men with normal percentage of sperm HOS test. The results of this study appeared that the patients with abnormal sperm HOS test have significant and positive correlations between sperm HOS test and percentages of sperm motility ($r= 0.565$; $P= 0.001$), progressive sperm motility ($r= 0.381$; $P= 0.003$) and normal sperm morphology of the human spermatozoa ($r= 0.299$; $P= 0.02$).

Discussion

The cigarette smoking has various detrimental effects on male reproductive system especially semen parameters (14). However, it was mentioned that the cigarette smoking adversely effects on human Leydig cell metabolism and function (15). 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 (16) and DNA damage (17). Furthermore, the cigarette contains large numbers of substances, including nicotine, carbon monoxide, carcinogens, mutagens and radioactive polonium (18). Moreover, inhalation of cigarette substances through active or passive smoking leads to absorption through the pulmonary vasculature and blood-borne circulation in the body.

Additionally, it was assessed that cigarette smoke substances could end up in the seminal plasma of smokers via various modes of diffusion and active transport (19). Also, chemical agents or mutagens may adversely affect hormonal control of spermatogenesis or may directly affect the Sertoli cells within seminiferous tubules in male smokers with disturbance of plasma membrane phospholipids asymmetry on sperm surface (20). A highly significant correlation was reported between the ability of male to achieve pregnancy and cigarette smoking (21). Also, 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 (22).

The direct effect of cigarette smoke on male germ cells are believable for both biologic and toxicological reasons and also indicated that smoking was associated with detrimental

effects on sperm concentration, motility, and morphology, and plasma membrane integrity with increase in disomic sperm and decrease in specific aspects of semen quality (23). It was noticed that semen parameters 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 (24).

The male reproduction can be impaired by a small but increasing number of environmental and occupational exposures (25). 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 (26). Those mechanisms may involve the hormonal control of spermatogenesis or may directly affect the germ and Sertoli cells within the seminiferous tubules (27). Although there is a number of studies have shown higher incidences of abnormally shaped sperm cells as well as decreased motility, sperm concentration, and HOS test score by decline plasma membrane integrity in men who smoke (28). Furthermore, fluctuations in male hormones (androgens) and other hormones responsible for the regulation of spermatogenesis and sex drive have been documented in male smokers (29).

From the results of the present study, it was concluded that the cigarette smoking have several impacts on sperm functions and integrity of plasma membrane, as well as sperm fertilizing ability. Further studies are recommended to assess the effect of cigarette smoking on DNA damage and embryo quality after IVF-ET.

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التأثير الضار لتدخين السكائر على متغيرات السائل المنوي وكفاءة غشاء النطفة البشرية البلازمي لمرضى العقم اللذين يجرون التمنية داخل الرحم

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الخلاصة:

صممت الدراسة الحالية إلى تقييم التأثير الضار لتدخين السكائر على متغيرات السائل المنوي و فحص كفاءة غشاء البلازما تحت الضغط التنافي الواطئ للنطف البشرية (HOS-test) ومعدلات الحمل بعد إجراء التمنية داخل الرحم (IUI) لمرضى العقم.

اشترك في هذه الدراسة مائة شخص يعاني من العقم تم تقسيمهم اعتماداً على تدخين السكائر الى مجموعتين المدخنين (العدد: 55) وغير المدخنين (العدد: 45). تم الحصول على عينة السائل المنوي وتقييم متغيرات النطف والتي شملت تركيز النطف والنسب المئوية لحركة النطف والحركة التقدمية للنطف والشكل الظاهري للنطف السوية وكفاءة غشاء البلازما تحت الضغط التنافي الواطئ للنطف البشرية اعتماداً على متغيرات منظمة الصحة العالمية (WHO) القياسية. تم تحضير نطف مرضى العقم باستخدام تقنية سباحة النطف للأعلى مباشرةً من خلال الحوض لفترة 30 دقيقة في 5% ثنائي أوكسيد الكربون وبدرجة 37 م°. أثبتت نتائج الدراسة الحالية ان متغيرات النطف والنسبة المئوية لفحص HOS test ونتائج التمنية داخل الرحم لمرضى العقم غير المدخنين أعلى من مرضى العقم المدخنين. هذا فضلاً عن نتائج متغيرات النطف والنسبة المئوية لفحص HOS test للمدخنين أكثر انحرافاً عن المدى الطبيعي لمتغيرات منظمة الصحة العالمية (WHO) القياسية من نتائج غير المدخنين. تم تقييم فروقات غير معنوية لفحص HOS-test بين غير المدخنين والمدخنين. لوحظ من نتائج هذه الدراسة ان تدخين السكائر له تأثيرات ضارة عديدة على وظائف النطف وكفاءة غشاء النطف البلازمي، هذا بالإضافة الى القدرة الاخصابية للنطف. نوصي باجراء دراسات لاحقة لتقييم تأثير تدخين السكائر على تضرر الدنا (DNA) ونوعية الأجنة بعد اجراء عمليات الاخصاب الخارجي ونقل الأجنة (IVF-ET).

Table (1): Parameters of seminal fluid analysis for infertile subjects classified according to smoking habit.

Parameters	Infertile patients		WHO criteria (1999)
	Non smokers *	Smokers **	
Semen volume (mL.)	2.81 ± 0.33	2.63 ± 0.22	≤ 2 ml
Semen liquefaction time (min.)	33.65 ±1.78	35.56 ±1.57	≤ 60 minutes
Semen Viscosity (cm.)	2.25 ± 0.23	2.45 ± 0.21	2 – 5 mL
Semen pH	7.62 ± 0.50	7.52 ± 0.32	7.2 – 8.0
Sperm concentration (×10 ⁶ sperm/mL)	52.11 ± 3.89	38.85 ± 3.23	≥ 20×10 ⁶ sperm/mL
Sperm Motility (%)	59.55 ±1.68	52.36 ±1.48	≥ 50 %

Progressive Sperm motility (%)	38.26 ±1.39	32.20 ±1.01	≥ 50 %
Sperm Agglutination (%)	12.33 ±0.03	15.36 ±1.07	≤ 10 %
Normal sperm morphology (%)	53.11±1.64	50.54 ±1.77	≥ 30 %
HOS test (%)	48.73 ± 1.45	42.00 ± 1.23	≥ 50 %

Values are Mean ± S.E.M

No. of non smoker patients=45

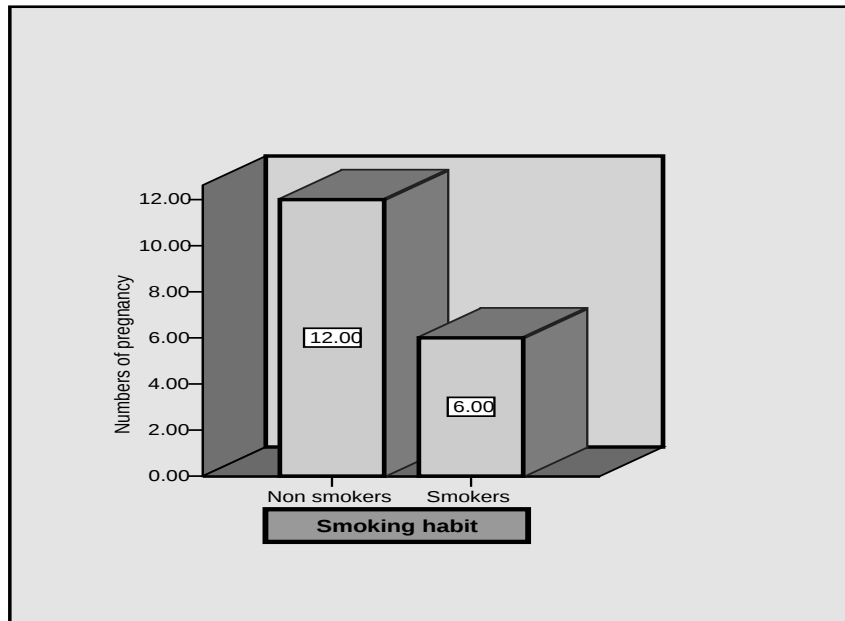
No. of smokers patients =55

Mean of age for non smokers patients (32.53 ± 1.09 years)

Mean of age for smokers patients (30.38 ± 0.80 years)

Standardization of smoking habit= heavy smoking habit (≥30 cigarette)

Figure (1): Outcomes of IUI for infertile couples classified according to smoking habit.



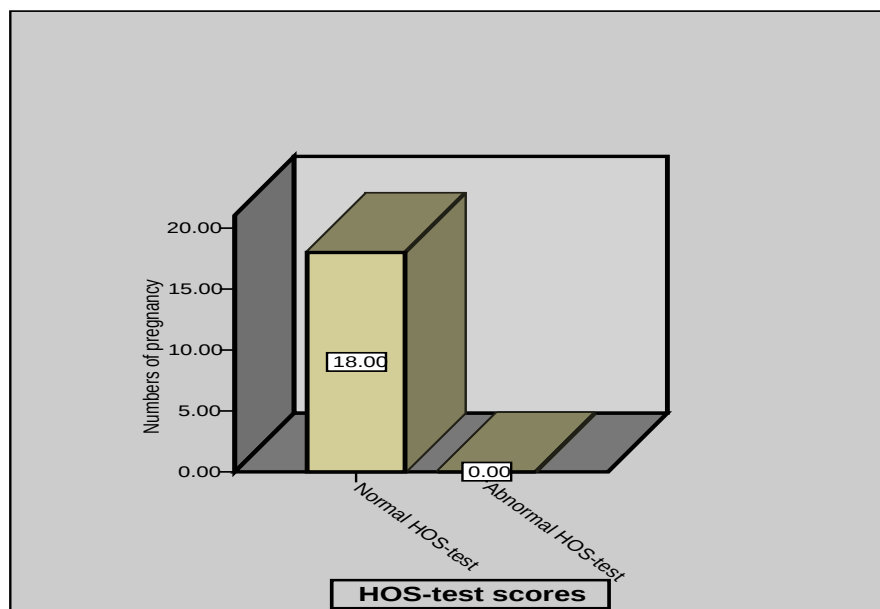
No. of non smoker subjects =45

No. of smoker subjects =55

Mean of age $\pm$ S.E.M for non smokers subjects (32.53 ± 1.09 years)

Mean of age $\pm$ S.E.M for smokers subjects (30.38 ± 0.80 years)

Figure (2): Outcomes of IUI for infertile couples classified according to scores of sperm HOS test.



Number of infertile subjects with normal HOS-test score=41

Number of infertile subjects with abnormal HOS-test score=59

Mean of age $\pm$ S.E.M for infertile subjects with normal HOS-test (31.95 ± 1.12)

Mean of age $\pm$ S.E.M for infertile subjects with abnormal HOS-test (30.93 ± 0.82)

