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A potential role of Nicotine on human sperm parameters *in vitro* .

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Abstract

This study aimed to investigate the effect of different nicotine concentrations (1,1.5 and 2) mg/ml on normal human sperm parameters in vitro . These concentrations were selected experimentally . Three incubation periods (30,45 and 60) minutes were used in this study .

The addition 1.5 or 2 mg/ml of nicotine to glucosaline media caused a significant decrease ($p < 0.001$) in sperm motility percent and grade activity compared to glucosaline alone or 1 mg/ml of nicotine when added to glucosaline , also a significant difference was noticed between 1.5 mg/ml and 2 mg /ml of nicotine when added to glucosaline in different incubation periods .

الخلاصة

هدفت الدراسة الى التحري عن تأثير التراكيز المختلفة من النيكوتين (1 و 1.5 و 2) ملغم / مل على معايير النطف البشرية السوية . اختيرت تلك التراكيز اعتمادا على التجربة واستخدمت ثلاث فترات حضن 30 و 45 و 60 دقيقة لجميع عينات النطف .

سببت إضافة 1.5 او 2 ملغم / مل من النيكوتين الى المحلول الفسيولوجي السكري انخفاضا معنويا ($p < 0.001$) في النسبة المئوية للنطف المتحركة ودرجة نشاط النطف مقارنة بمجموعة السيطرة (المحلول الفسيولوجي السكري لوحده) او بالتركيز 1 ملغم / مل من النيكوتين بعد إضافته الى المحلول الفسيولوجي السكري . كذلك لوحظ فرق معنوي بين التركيزين 1.5 و 2 ملغم / مل عندما أضيفت الى المحلول الفسيولوجي السكري ولفترات الحضن المختلفة .

Introduction

Cigarette smoking is one of lifestyle factor that contains more than 3000 different chemical compounds, such as nicotine, nitrosamine , polycyclic aromatic hydrocarbons, cadmium and carbonmonoxide. Some of these chemicals are genotoxic by entering the blood circulation of the testes and have direct cytotoxic effect on spermatozoa [Bos and Henderson, 1984], and may cause genetic damage on it [Evans *et al.*, 1981], then may disturbing normal testicular steroidogensis and spermatogenesis in male smokers [Shaarawy and Mahmoud, 1982; Klaiber and Broverman, 1988] . Also cigarette was impaired sexual activity and might contribute to impotence at any age [Nakagawa *et al* ., 1990] , decrease the sperm motility [Rubes *et al* ., 1998] and morphology [Alwachi *et al.*, 1986 & Wong *et al* ., 2000] . The cadmium exposure was mostly increased due to smoking habit, which adversely affect on acrosome reaction insuficiency , abnormal sperm morphology [Benoff *et al.*, 1997] and decrease in sperm motility [Telisman *et al.*, 2000] . So that this study was aimed to investigate the effect of different nicotine concentrations on normal human sperm parameters in vitro .

Materials and Methods

- Semen collection and processing

Ten semen samples were obtained by masturbation from ten fertile men after 3 days of sexual abstinence .The specimens were allowed to liguefy at 37° C for 20-30 minutes . The mean data of the semen parameters were estimated , including : sperm concentration , sperm motility percent grade activity and abnormal sperm morphology percent . The values of these parameters considered as sperm parameters before activation .

-*in vitro* sperm activation technique and semen incubation with nicotine.

Two ml of each semen sample was divided into four equal splits . and placed separately in four centrifuged tubes. Each 0.5 ml of semen was mixed with 1 ml of glucosaline media supplemented with 20 % inactive maternal serum [Al-Taee, 1994] . The mixture was centrifuged at 2000 rpm for 5 minutes, the supernatant was

discarded and the final pellet in four tubes were overlayered with glucosaline media , glucosaline+1 mg/ml of nicotine , glucosaline+1.5 mg/ml of nicotine and glucosaline + 2 mg/ml of nicotine respectively , these concentrations were chosed expermentaly . The tubes contain samples kept in the incubator at 37° C .

After 30 , 45 and 60 minutes of incubation , adrop of top part of glucosaline alone and glucosaline with nicotine were aspirated by pipette and examined to evaluate sperm parameters after activation . The results were analyzed by using analysis for variance (ANOVA) and LSD to indicate the significancy [Scheffer , 1980]

Results

The result of in vitro sperm activation of normal semen specimens by using glucosaline alone or with 1 , 1.5 and 2 mg/ml of nicotine showed a significant decrease ($p<0.001$) in sperm concentration and abnormal sperm morphology percent compared to those values before activation after all incubation periods (Table 1,2 and 3). While sperm motility percent and grade activity were significantly decreased ($p < 0.001$) by using 1.5 and 2 mg/ml only compared to those before activation, control and glucosaline + 1 mg/ml of nicotine , as well as there is a significant difference between 1.5 and 2 mg/ml of nicotine after 30 minutes of incubation period (Table 1) .

Table 1 : Normal human sperm parameters before and after activation by glucosaline medium with different nicotine concentrations for 30 minutes of incubation period .

Sperm parameters	Before treatment (mean ±SD)	After treatment (mean ± SD)			
		glucosaline	Glucosaline+1 mg/ml nicotine	Glucosaline+1.5 mg/ml nicotine	Glucosaline+2 mg/ml nicotine
Sperm concentration ($\times 10^6$)	a 84.40 ± 19.39	b 20.40 ±6.16	b 19.10±8.86	b 17.10± 8.02	b 17.00± 7.48
Sperm Motility percent	a 61.00 ±5.38	a 73.00 ± 6.78	a 67.00± 11.10	b 38.00± 24.96	c 19.50± 16.57
Grade activity	a 3.19± 0.21	a 3.75 ± 0.30	a 3.58± 0.39	b 2.00±1.30	c 1.08±1.03
Abnormal sperm morphology percent	a 30.39± 6.71	b 14.74 ± 4.75	b 16.70± 4.76	b 14.20± 5.60	b 13.66± 5.11

Number of semen donar = 10
P < 0.001 significant difference
Different letters indicate for significancy.

The incubation for 45 and 60 minutes caused significant decrease ($p < 0.001$) in sperm motility percent and grade activity when incubated with 1.5 and 2 mg/ml of nicotine with glucosaline compared to glucosaline alone and glucosaline+1 mg/ml of nicotine. Also there are significant decrease ($p < 0.001$) in those two above parameters by using 2 mg/ml of nicotine compared to 1.5 mg/ml (Tables 2 and 3) .

Table 2: Normal human sperm parameters before and after activation by glucosaline medium with different nicotine concentrations for 45 minutes of incubation period.

Sperm parameters	Before treatment (mean ±SD)	After treatment (mean ± SD)			
		glucosaline	Glucosaline+1 mg/ml nicotine	Glucosaline+1. 5 mglml nicotine	Glucosaline+2 mg/ml nicotine
Sperm concentration (x 10^6)	a 84.40± 19.39	b 19.60± 9.67	b 20.40± 9.93	b 20.20± 9.64	b 15.80± 10.11
Sperm motility percent	a 61.00 ±5.38	b 84.50± 6.78	b 79.00±7.74	c 36.50± 23.45	d 18.50±16.84
Grade activity	a 3.19 ± 0.21	b 4.05 ± 0.34	b 3.85±0.31	c 1.96± 1.26	d 1.00±1.05
Abnormal sperm morphology percent	a 30.39 ± 6.71	b 14.53 ± 6.44	b 13.65±6.86	b 14.71± 5.22	b 15.06± 5.79

Number of semen donar = 10
P<0.001 significant difference
Different letters indicate for significancy .

Table 3: Normal human sperm parameters before and after activation by glucosaline medium with different nicotine concentrations for 60 minutes of incubation period.

Sperm parameters	Before treatment (mean ±SD)	After treatment (mean ± SD)			
		glucosaline	Glucosaline+1 mg/ml nicotine	Glucosaline+1. 5 mg/ml nicotine	Glucosaline+2 mg/ml nicotine
Sperm concentration (x 10^6)	a 84.40±19.39	b 20.20± 12.55	b 20.80±12.66	b 18.30± 11.38	b 14.90± 12.03
Sperm motility percent	a 61.00 ±5.38	b 89.50± 7.88	b 84.00± 6.99	c 34.50±22.29	d 18.00± 15.31
Grade activity	a 3.19 ± 0.21	b 4.36 ± 0.45	b 4.10± 0.29	c 1.95±1.26	d 0.97± 0.91
Abnormal sperm morphology percent	a 30.39 ±6.71	b 12.30±4.40	b 16.58± 6.23	b 16.03± 5.54	b 15.71± 4.99

Number of semen donar= 10
P<0.001 significant difference
Different letters indicate for significancy .

Discussion

The results of this study revealed to significant decrease is sperm motility percent and grade activity when the spermatozoa were incubated with glucosaline supplemented with 1.5 and 2 mg/ml of nicotine compared to glucosaline alone or with 1 mg/ml of nicotine. These results agreement with Crandall *et al* (1989) study which are showed that incubated sperms with 100 , 1000 and 5000 mg/ml of nicotine in BWW medium did not affected on sperm motility due to the low concentrations of nicotine.

The result showed that decrease in sperm motility percent and grade activity after adding 1.5 or 2 mglml of nicotine to glucosaline will be remain up to 60 minutes of incubation, this effect may be due to the action of nicotine on ultrastructure of the flagellum and consequently the motility and progressive motility of spermatozoa [Zavos et al., 1999], or may be have direct effects of destruction on the lipid bilayer of the cellular membrane with higher concentrations.

Other study suggest that smoking in men did not impair sperm quality or reduce fertility [Hughes and Brennan, 1996] and there is no significant differences between semen quality of non smokers, light/ moderate smokers, and heavy smokers [Godfrey, 1981]. Although nicotine is prernt in alarger amount in the semen of smoking men comp-
aring with non- smoking men [Zenzes *et al.*, 1996]. This may be due to the ability of nicotine to pass through blood– testicular barrier and can be eliminated from the male genital tract together with ejaculate , by this way sperms can be maintained from the toxicity of some compounds [Piasecka et al., 1995; Sprando et al ., 1996; Sprando et al., 1997; Sprando et al., 1998]. Other study confirmed that the macrophages in interstitial tissue of testes can protect the seminiferous epithelium against the cytotoxic effects of some compounds [Rozewicka *et al.*, 1995] or rapid degradation of mitochondria or other cell structures should be occurred and increased membranes formation which are exploited for evacuating the toxic compound in the cells [Wiszniewska *et al.*, 1998] .

The treatment of spermatozoa with nicotine as in this study caused harmful effects may be because the exposure to nicotine was in vitro and in this case there is no defence line protect the spermatozoa from the toxicity of nicotine like in vivo exposure.

The results showed that insignificant differences in recovery sperm concentration and abnormal sperm morphology percentage in different treatments compared to control, this may be due to that sperm concentration and morphology was completely determined in vivo, and usually in vitro studies affected in semen quality only.

It was concluded that sperm motility parameters were adversely affected by the addition 1.5 or 2 mg/ml of nicotine to normal semen samples incubated with glucosaline medium, and these effects will be increased with increasing of incubation period from 30 minutes to 60 minutes.

References :

Al-Taee , H.A.J. (1994). Sperm activation in intrauterine insemination : the effect of serum concentrations in culture media on sperm activation potential *in vitro.* MSC thesis by Al-Taee , H.A.J. , PP : 92-95 .

Alwachi , S.N. ; Al-Kobaisi , M.F. & Mahmoud , F.A. (1986) . Possible effect of nicotine on the spermatogenesis and testicular activity . JBSR.Vol. 17 (3): 185 – 194 .

Benoff, S.; Hurley, I. R.; Barcia, M.; Mandel, F. S.; Copper, G. W. and Hershlay, A. (1997). Apotential role for cadmium in the etiology of varicocele– associated infertility. Fertility and Sterility; 67: 336– 347.

Bos, R. P. and Henderson, P.T. (1984). G enotoxic risk of passive smoking. Rev Environ Health; 4: 161– 178. cited by Wong, Y.; Thomas, C. M.; Merkus, H .M.; Zielhuis , G. A.; Doesburg, W. H. and Theunissen, R. P. (2000). Cigarette smoking and the risk of male factor subfertility: minor association between nicotine in seminal plasma and semen morphology. Fertility and Sterility; 74: 930– 935.

Crandall, L. A . ; Hensleigh , H . C . and Phipps , W . R . (1989) . Effect of nicotine on in vitro human sperm penetrability throughcervical mucus and motility parameters . Fertility and Sterility ; 51 : 722 – 724 .

Evans , H . J . ; Fletcher , J . ; Torrance , M . and Hargreave , T . B . (1981) . Sperm abnormalities and cigarette smoking . Lancet ; 21:627 – 629 .

Godfrey , B. (1981) . Sperm morphology in smokers in smokers . Lancet ; 25 : 948 – 949 .

Hughes , E.G. and Brennan , B.G . (1996) . Does cigarette smoking impair natural or assisted fecundity ? . Fertility and Sterility ; 66 : 679 – 689 .

K laiber, E. and Broverman, D. M. (1988) . Dynamics of estrad –oil and testerone and seminal fluid index in smokers and nonsmokers . Fertility and Sterility; 50 ; 630 – 634 .

Nakagawa , S ; Watanaba , H . ; Ohe , H. and Nakao , M . (1990) . Sexual behavior in Japanese males relating to area occupation, smoking, drinking and eating habits . Andrologia ; 22 : 21 – 28 .

Piasecka, M.; Rozewicka , L.; Laszczynska, M. and Marchlewicz , M. (1995) . Electron – dense deposits in epididymal cells of rats chronically treated with lead acetate pb(II) . Folia Histochemica Cytolobiologica ; 33 : 89 – 94 .

Rozewicka , L.W.; Marchlewicz , M.; Wiszniewska , B.B. and Piasecka , M. (1995). The ultrastructure of the testis in the rats after Long – term treatment with Lead acetate . Andrologia ; 28 : 97 – 102 .

Rubes, J.; Lowe , X . ; Moore , D.; Perreault , S. ; Slott , V.; Evenson , D. ; Selevan , S.G . and Wyrobek , A.J. (1998) . Smoking cigarette is associated with increased sperm disomy in teenage men . Fertility and Sterility; 70: 715– 723 .

Scheffer , W. C . (1980) . Statistics for the biological science .2 edition . Scheffer , W . C . (ed) . Addison westry publishing company . California , London . Amesterdam .

Shaarawy , M . and Mahmoud , K . Z . (1982) . Endocrine profile and semen characteristics in male smokers . Fertility and Sterility ; 38 : 255 – 257 .

Sprando , R L. ; Black , T.N.; Ames , M.J.; Rorie , J.I. and Collins , T.F. X. (1996). Effect of intratesticular injection of sodium fluoride on spermatogenesis . Food and Chemical Toxicology ; 34 : 377 – 384 .

Sprando , R .L .; Collins , T .F . X . ; Black , T ; Olejnik , N .+ Rorie , J . (1998) . Testing the potential of sodium fluoride to affect spermatogenesis : a morphometric study . Food and Chemical Toxicology ; 36 : 1117 – 1124 .

Sprando , R. L. ; Collins , T . F . ; Black , T . ; R orie , J . + Odonnell , M . (1997) . Testing the potential of sodium fluoride to affect spermatogenesis in the rat . Food and Chemical Toxicology ; 35 : 881 – 890 .

Telisman, S.; Cvitkovic , P.; J urasovic , J. ; Pizent , A.; Gavella , M. Rocic , B. (2000). Semen quality , and reproductive endocrine function in relation to biomarkers of lead , cadmium , Znic , and copper in men . Environmental Health Perspectives ; 108 : 45 – 53 .

Wiszniewska , B.; Marchlewicz , M.; Piassecka , M.; Rozewicka , L. W. Amawi, M.S. (1998). Phospholipd content and lamellar structures in the epididymal epithelial cells of rats treated chronically with lead acetate pb (II) Folia biologica ; 46 : 215 – 224 .

Wong , W.Y.; Thomas , C.M.; Merkus , H.M.W. Zielhuis, G.A. ; Doesburg, W.H . Theunissen , R.P.M. (2000) .Cigarette smoking and the risk of male factor subfertility : minor association between cotinine in seminal plasma and semen morphology . Fertility and Sterility ; 74 : 930 – 935 .

Zavos , P. M . ; Zarmakoupis , C.N . Zarmakoupis- Zavos , P.N . (1999). The impact of cigarette smoking on human reproduction : its effect on female and male fecundity . Middle East Fertility Society Jounal ; 4 : 94 – 101 .

Zenxes , M.T. ; Reed , T.E.; Wany, P . Klei, J . (1996). Cotinine , a major metabolite of nicotine , is detectable in follicular fluids of passive smokers in invitro fertilization therapy . Fertility and Sterility ; 66 : 614 – 619 .