ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Endocrinal dysfunction of Hypothalamo Pituitary Gonadal Axis in seminal plasma of Cigarette Smoking Males.

Sherif El-Kannishy[1,2]*, Aalaa Aboelnour[3], Waleed Barakat[1,4], Mohamed S. Abdel-Maksoud[1], and Abdullah A. AL Yousef[5].

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Tabuk, Saudi Arabia.
[2]Department of Analytical Toxicology - Emergency Hospital, Faculty of Medicine, University of Mansoura, Egypt.
[3]Department of Clinical Pathology, Faculty of Medicine, University of Mansoura, Egypt.
[4]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Zagazig University, Egypt.
[5]Department of Dermatology, Faculty of Medicine, University of Tabuk, Saudi Arabia.

ABSTRACT

Tobacco smoke induces poor semen quality and male infertility. Therefore, we assess the effect of cigarettes on hormones of seminal plasma. Semen samples were obtained from forty tobacco married smoker men and forty strict married non-smoker men. Semen samples were subjected to: i) Analysis by a computer assisted semen analyzer. ii) Analysis for testosterone, follicular stimulating hormone and luteinizing hormone. Infertile smokers and non-smokers had significantly higher levels of FSH and LH compared to fertile smokers and non-smokers respectively. Level of testosterone was significantly lower in infertile smokers and non-smokers compared to fertile smokers and non-smokers. In conclusion, our study has shown that infertile smoking men have reduced semen quality and altered hormonal level than infertile nonsmokers.

Keywords: Infertility, Testosterone, Follicular stimulating hormone, seminal plasma.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Infertility affects 10%–15% of couples worldwide and the male factor accounts for about half of infertility problems [1]. In addition, the male reproductive system is known to be highly sensitive to many chemicals and drugs which have been found to pose adverse effects on male reproductive capacity under certain conditions [2]. One of the toxicants that have been reported to have detrimental effects on male reproductive health is cigarette smoke (CS) [3].

Although cigarette smoking is a widely recognized health hazard and a major cause of mortality [4] killing 1 in 10 adults worldwide [5], yet, around one third of the world's population over 15 years old smokes daily [6]. The highest prevalence of smoking is observed in young males during their reproductive age, as 46% of smokers (SM) are aged between 20 and 39 years [4, 7].

Cigarette smoke (CS) contains a large number of substances including nicotine, carbon monoxide and recognized carcinogens and mutagens such as radioactive polonium, cadmium, benzo(a)pyrene, dimethylbenz(a)anthracene, dimethylnitrosamine, naphthalene and methnaphthalene [8].

Inhalation of CS, either through active or passive smoking leads to absorption of these agents through vasculature and blood-borne circulation throughout the body [9]. It is also possible that CS-derived substances could end up in the seminal plasma (SP) [4].

Cigarette smoking is the most important known preventable cause of death all over the world [9]. Despite worldwide antismoking campaigns, cigarette smoking is very common [7, 10] and smoking still presents one of the most common addictions [11].

Several studies have demonstrated that certain chemicals present in cigarette smoke can adversely affect male fertility [6], [12-14] by causing substantial negative effects on sperm production, motility, and morphology [7].

These effects can be attributed to the disturbance of the hypothalamus–pituitary–gonadal axis by the high levels toxic substances contained in cigarettes or by the increased apoptotic process that takes place during spermatogenesis [15].

The reproductive axis (the hypothalamic–pituitary– gonadal axis (HPG)) in males is composed of 3 major components; the hypothalamus, the pituitary gland and the testis. The regulation of such axis affects the steroid sensitive prostate and the penis. The hypothalamus is the main integration center of the HPG axis. The hypothalamus is responsible for the production of the gonadotropin-releasing hormone (GnRH) which is transported to the adenohypophysis of the pituitary gland by a short portal venous system resulting in the synthesis and release of gonadotropic hormones (luteinizing hormone-LH and follicle stimulating hormone-FSH. On normal basis, the axis works in a unique regulated manner to produce the required steroids into the circulation that are essential for male sexual development, in addition to sexual functions and fertility. Hypogonadotropic hypogonadism is declared when low serum testosterone levels or abnormal low-normal serum LH is observed. An abnormality in hypothalamus or the pituitary gland is mainly the cause of such clinical manifestation [16].

Chronic smoking can cause the impairment of fertility [11, 17]. A consistent number of studies have claimed that cigarette smoking is correlated with alterations in sperm quality [4], [18-23]. However, other studies failed to find any impact of cigarette smoking on semen parameters [24-27].

Despite the various harmful effects of smoking on male fertility, most male smokers are still fertile but have a higher risk of subfertility or infertility [14].

The current study was designed to investigate the possible relationship between smoking and male infertility in terms of semen quality and hormonal levels among groups of fertile and infertile smoking and non-smoking men.

ISSN: 0975-8585

RESEARCH METHOD

This study was carried out upon 80 adult males' smokers attending the outpatient clinics of andrology, Mansoura University Hospitals, Egypt. Informed consents were taken from all partners.

All patients were subjected to:

a) **Personal history**: including Name, age, job, and address and special habits mainly cigarette Smoking (duration of smoking, number of cigarettes /day). The smokers included in the study were those who smoked cigarettes on a regular basis for at least 1 year. The men who never smoke or stopped smoking one year prior to examination were considered nonsmokers [28].

b) **Fertility history:** Infertile defined as failure to conceive within one year of regular unprotected intercourse. The investigated subjects were primary idiopathic infertility since more than five years of marriage. The participants had clinically normal epididymis and ductus deferens. Then the female partner was evaluated for infertility factors and they had no inducing factor for infertility, normal menstrual and secondary sex characters as well as normal reproductive investigations for ovulation and ovarian, tubal and uterine clinical status.

c) **Medical history of diseases with possible adverse effect on fertility:** None of them gave a past history of genital infection or trauma; chronic systemic disease or long term of medical treatment.

Patients were divided in to two main groups:

Group I

Forty tobacco smoking men: All of them were married for more than five years. Half of them was primary infertile. Each man smoked from 15 to 30 cigarettes per day for more than one year.

Group II

Forty strict tobacco non-smoking men: Half of them was primary infertile after > five years marriage with unprotected intercourse.

Exclusion criteria

Patient with history of diseases or factors with possible adverse effect on fertility were excluded, none of them gave a past history of genital infection, trauma, chronic systemic disease, long-term medications or chemical exposure.

Semen analysis

Sample collection and delivery

The samples were collected after a minimum of 48 hours and not longer than 5 days of sexual abstinence. The samples were obtained by masturbation and ejaculated into a clean, wide-mouthed, plastic container. The containers were warmed to minimize the risk of cold shock.

Sample examination

After liquefaction (within 1.0 hour from collection), each semen sample was subjected for analysis [29] by a computer assisted semen analyzer (Weili Color Sperm Analysis System: ALJY-9000, China).

ISSN: 0975-8585

Seminal plasma examination

Plasma of each semen sample was separated after whole semen centrifugation at 5000/rpm for 10 minutes and frozen at -70ºC till being subjected to the following determinations hormonal levels of each of the following:

LH and FSH levels (uIU/ml) [30].
Total testosterone levels (ng/dl) [31].

By solid phase, 2 site chemiluminescent immunometric assay. [IMMULITE 1000: (Diagnostic Products Corporation (DPC), corporate offices: 5210 pacific concourse drive, Los Angeles, CA 90045 – 6900, USA].

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Graphpad prism software v.5 (GraphPad Software, Inc., La Jolla, CA, USA). The inter-group variation was measured by one-way analysis of variance (ANOVA) followed byTukey's post-hoc test.The level of significance was read at the probability value $p<0.05$.

RESULTS

Differences in semen parameters

In the current study, smokers (fertile and infertile) had impaired semen quality compared to the corresponding non-smokers groups (fertile and infertile); infertile smokers had significantly smaller ejaculate volume compared to fertile smokers and infertile non-smokers (2.1 vs 2.9 and 2.8 ml respectively)(table 1).

Table 1: Differences in semen parameters among fertile and infertile smoker and non-smoker groups. Data are shown as mean ± SEM (n = 15).

Variables	Non-smoker		Smoker	
	Fertile	Infertile	Fertile	Infertile
Volume (ml)	3.1 ± 0.18	2.8 ± 0.24	2.9 ± 0.16	2.1 ± 0.13 *#@
Count (million/ml)	65.8 ± 4.1	49.9 ± 3.3 *	38.0 ± 2.1 *#	32.1 ± 2.7 *#@
Viability %	67.7 ±4.3	40.4 ± 2.5 *	54.4 ± 3.3 *#	28.6 ± 2 *#@
Progressive motility %	80.3 ± 4.9	41.6 ± 2.7 *	62.6 ± 5.1 *#	33.6 ±2 *#@
Normal morphology %	61.5 ±3.4	31.8 ± 2.3 *	53.6 ± 3.7 *#	23.3 ± 2.4 *#@
Round head %	17.9 ± 1.5	31.5 ±1.4 *	23.3 ± 1.6 *#	40.5 ± 2.5 *#@

** Significantly different from the non-smoker fertile group, # significantly different from the non-smoker infertile group, and @ Significantly different from the smoker fertile group at p>0.05 using ANOVA followed by Tukey's post-hoc test.*

In addition, the sperm count in the infertile non-smokers was significantly less compared to the fertile non-smokers (49.9 vs 65.8 million/ml), similarly, the sperm count was significantly less in infertile smokers compared to fertile smokers (32.1 vs 38 million/ml). It was also evident that smokers (fertile or infertile) had significantly less sperm count than the corresponding non-smoker groups (fertile or infertile).

Infertile non-smokers had less sperm viability compared to fertile non-smokers (40.4 vs 67.7 %), also infertile smokers had less sperm viability compared to fertile smokers (28.6 vs 54.4 %). In addition, the sperm viability was significantly less in smokers (fertile or infertile) compared to the corresponding non-smokers (fertile or infertile).

Our results also showed that the sperm progressive motility was significantly less in the infertile non-smokers compared to fertile non-smokers (41.6 vs 80.3 %), also, the infertile smokers had less semen progressive motility compared to the fertile smokers (33.6 vs 62.6 %). Similarly, smokers (fertile or infertile) had significantly less sperm progressive motility than the corresponding non-smokers (fertile or infertile) groups.

ISSN: 0975-8585

The previous changes were accompanied by alterations in sperms normal morphology, where, the infertile non-smokers had less normal morphology compared to the fertile non-smokers (31.8 vs 61.5 %) and also the infertile smokers had had less normal morphology compared to the fertile smokers (23.3 vs 5.6 %). In addition, smokers (fertile or infertile) had significantly less normal sperm morphology than the corresponding non-smoker groups (fertile or infertile).

The most evident alteration observed in sperm morphology was the round heads and more sperms in the infertile non-smokers group had round heads compared to the fertile non-smokers (31.5 vs 17.9 %), in addition, more sperms from the infertile smokers had round heads compared to the fertile smokers (40.5 vs 23.3 %). Moreover, smokers (fertile or infertile) had significantly more sperms with round heads than the corresponding non-smoker groups (fertile or infertile).

Differences in hormonal level

It was obvious that infertile non-smokers had significantly higher level of FSH compared to the fertile non-smokers (7.3 vs 6 mIU/ml), in addition, infertile smokers had significantly higher level of FSH compared to the fertile smokers (7.8 vs 6.3 mIU/ml) **(Figure 1a).**

Similarly, infertile non-smokers had significantly higher level of LH compared to the fertile non-smokers (6.8 vs 5.4 mIU/ml), and infertile smokers had significantly higher level of LH compared to the fertile smokers (5.2 vs 4.1 mIU/ml). Moreover, the level of LH was significantly higher in fertile smokers compared to fertile non-smokers **(Figure 1b).**

On the other hand, the level of testosterone was significantly lower in infertile non-smokers compared to fertile non-smokers (326.6 vs 491.4 ng/dl). Also, infertile smokers had less testosterone level compared to fertile smokers (378 vs 509.2 ng/dl) **(Figure 1c).**

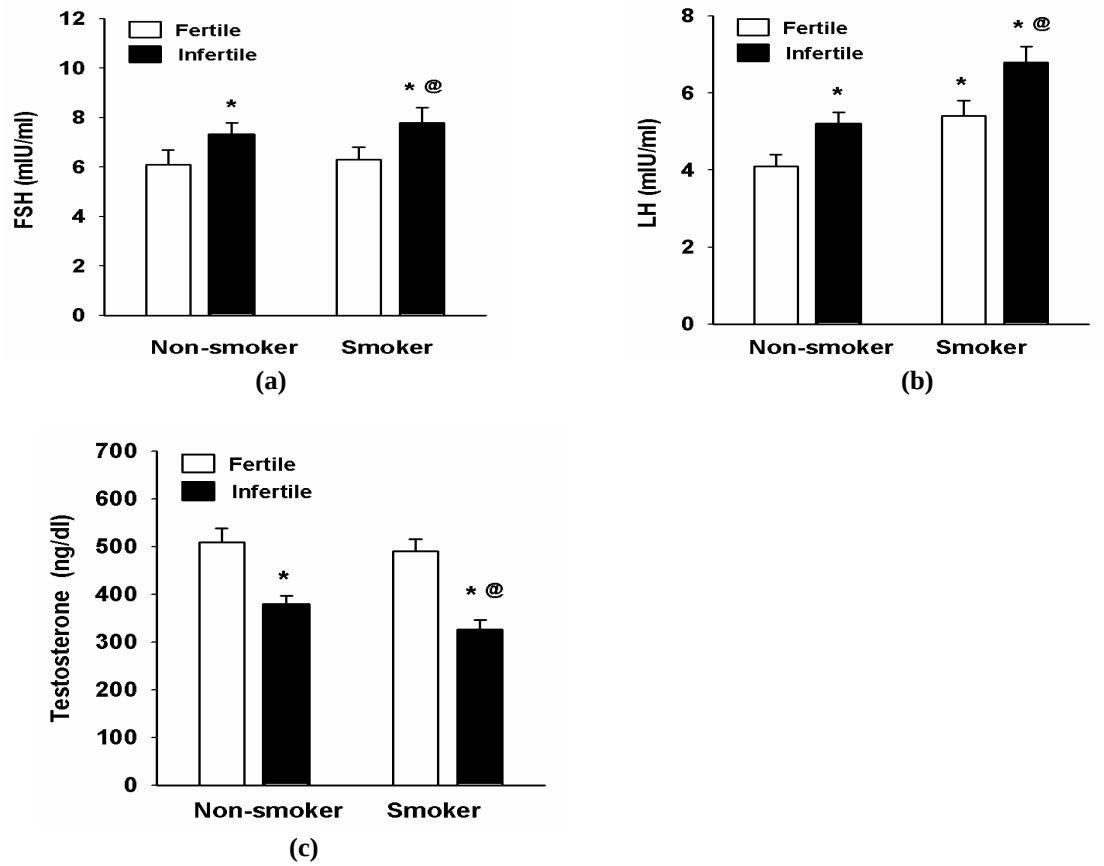

Figure 1: Differences in hormonal level: a) FSH, b) LH and c) testosterone among fertile and infertile smoker and non-smoker groups. Data are shown as mean ± SEM (n = 15). * Significantly different from the non-smoker fertile group, # Significantly different from the non-smoker infertile group, and @ Significantly different from the smoker fertile group at p>0.05 using ANOVA followed by Tukey's post-hoc test.

DISCUSSION

Infertility is a major health issue among couples of childbearing age and approximately half of known causes of primary infertility are attributed to male factor [32].

However, a specific male disorder may not always be identified and for this reason exposure to a small but increasing number of environmental or occupational toxic agents [4], estrogenic pollutants [33], obesity, and lifestyle factors such as cigarette smoking and alcohol intake [34] or have drawn much attention.

The effect of smoking on semen quality is difficult to discern due to confounding effects such as the smoking habits of a partner, environmental factors, and genetic background [26].
Although the exact mechanisms by which smoking exerts its harmful effect on semen parameters are not fully understood, a possible explanation is the direct toxic impact of accumulated nicotine (or other chemical components) in the epithelium of the male germ line [35, 36].

The current study has shown that, infertile smokers had significantly smaller ejaculate volume compared to fertile smokers and infertile non-smokers.

The infertile non-smokers had less sperm count, percent of sperm viability, progressive motility and normal morphology compared to the fertile non-smokers was significantly less compared to the fertile non-smokers. Similarly, infertile smokers had less sperm count, percent of sperm viability, progressive motility and normal morphology compared to the fertile smokers. In addition, more sperms in the infertile non-smokers and infertile smokers had round heads compared to the corresponding fertile groups (non-smoker or smoker).

Moreover, all these changes were more prominent in smokers than the non-smokers which imply that smoking resulted in a significant decrease in semen quality in both fertile and infertile smoker groups compared to the corresponding non-smoker groups.

Similar effects of smoking on ejaculate volume, sperm count, sperm viability, progressive motility and sperm normal morphology were previously reported [2, 7, 14, 15, 17, 20, 25, 28] [37-54].

On the other hand, some studies have reported nearly similar values for semen parameters between smokers and nonsmokers [24-26].

The current study has shown that infertile men (smokers or non-smokers) had significantly higher levels of FSH and LH compared to the corresponding fertile groups (smokers or non-smokers). This was accompanied by a decrease in testosterone level in infertile men (smokers or non-smokers) compared to the corresponding fertile groups (smokers or non-smokers). However, the differences between infertile smokers and infertile non-smokers were not significantly different.

Tobacco consumption has recently been documented to act as an endocrine disruptor on the male hormone profile, specifically on LH, testosterone, and prolactin levels [55].

Some studies have indicated increased level of total testosterone in smokers [55-57]. Meanwhile, other studies reported that the levels of testosterone did not differ in smokers [14, 58], whereas other studies found reduced testosterone levels [59, 60]. These controversies with the effect of cigarette smoking and reproductive hormones in men might be attributed to the relative small sample size and the widespread confounding factors in these observational studies [14].

SUMMARY AND CONCLUSION

Our study has shown that infertile men (smoker or non-smoker) have reduced semen quality and altered hormonal level. Additionally, smokers (fertile or infertile) had more evident reduction in semen quality while their hormonal level was not significantly different from the corresponding non-smoker groups. This implies that smoking might mediate infertility by altering the hormonal levels. Accordingly, programs that encourage smoking cessation should be highly adopted by different health organizations allowing targeted therapies and more effective approaches for smoking cessation to preserve the reproductive health.

ISSN: 0975-8585

ACKNOWLEDGEMENTS

The authors would like to acknowledge financial support for this work from the Deanship of Scientific Research (DSR), University of Tabuk, Tabuk, Saudi Arabia, under grant number S/1436/0069.

REFERENCES

[1] Bashamboo AB, Ferraz-de-Souza, B, Lourenço D, Lin L, Sebire NJ, Montjean D, Bignon-Topalovic J, Mandelbaum J, Siffroi JP, Christin-Maitre S, Radhakrishna U, Rouba H, Ravel C, Seeler J, Achermann JC, McElreavey K. Am J Hum Genet 2010; 87: 505-12.
[2] Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. J Reprod Infertil 2011; 12: 201-7.
[3] Mohamed M, Sulaiman SA, Sirajudeen KN. Toxicol Ind Health 2012; 29: 264-71.
[4] Arab M and Moshtaghi H. Andrologia 2005; 37: 119-24.
[5] Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, Wollum A, Sanman E, Wulf S, Lopez,AD, Murray CJ, Gakidou E. 2014; 311: 183-92.
[6] Zenzes M.T. Hum Reprod Update 2000; 6: 122-31.
[7] Kunzle R, Mueller MD, Hänggi W, Birkhäuser MH, Drescher H, Bersinger NA. Fertil Steril 2003; 79: 287-91.
[8] Stillman RJ, Rosenberg MJ, Sachs BP. Fertil Steril 1986; 46: 545-66.
[9] Santos EP, López-Costa S, Chenlo P, Pugliese MN, Curi S, Ariagno J, Repetto H, Sardi M, Palaoro L, Mendeluk G. Andrologia 2011; 43: 431-5.
[10] Oyeyipo IP, Raji Y, Bolarinwa AF.. J Hum Reprod Sci 2013; 6: 40-4.
[11] Hruskovicov H, Dušková M, Simůnková K, Hill M, Pospíšilová H, Rácz B, Králíková E, Vondra K, Stárka L. Physiol Res 2013; 62: 67-73.
[12] Sepaniak S, Forges T, Gerard H, Foliguet B, Bene MC, Monnier-Barbarino P. Toxicol 2006; 223(1-2): 54-60.
[13] Condorelli RA, La Vignera S, Giacone F, Iacoviello L, Vicari E, Mongioi,' L, Calogero AE. Int J Immunopathol Pharmacol 2013; 26: 739-46.
[14] Dai JB, Wang ZX, Qiao ZD. Asian J Androl 2015; DOI: 10.4103/1008-682X.150847.
[15] Anifandis G, Bounartzi T, Messini CI, Dafopoulos K, Sotiriou S, Messinis IE. Arch Gynecol Obstet 2014; 290: 777-82.
[16] Jin JM and Yang WX. Gene 2014; 1: 15-25.
[17] Kumar R. Indian J Cancer 2010; 47: 107-8.
[18] Ozgur K, Isikoglu M, Seleker M, Donmez L. Arch Gynecol Obstet 2005; 271: 109-12.
[19] Robbins WA, Elashoff DA, Xun L, Jia J, Li N, Wu G, Wei F. Cytogenet Genome Res 2005; 111: 371-7.
[20] Gaur DS, Talekar M, Pathak VP. Effect of cigarette smoking on semen quality of infertile men. Singapore Med J 2007; 48(2): 119-23.
[21] Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod 2007; 22(1): 188-96.
[22] Pasqualotto FF, Umezu FM, Salvador M, Borges EJr, Sobreiro BP, Pasqualotto EB. Fertil Steril 2008; 90(2): 278-83.
[23] Elshal MF, El-Sayed IH, Elsaied MA, El-Masry SA, Kumosani TA. Clin Biochem 2009; 42(7-8): 589-94.
[24] Chohan KR and Badawy SZ.. Int Braz J Urol 2010; 36: 60-5.
[25] Collodel G, Capitani, S, Pammolli A, Giannerini V, Geminiani M, Moretti E. J Androl 2010; 31: 108-13.
[26] Yu BJ, Chen J, Liu D, Zhou H, Xiao W, Xia X, Huang Z. Biol Reprod 2013; 89: 5.
[27] de Jong AM, Menkveld R, Lens JW, Nienhuis SE, Rhemrev JP. Andrologia 2014; 46: 112-7.
[28] Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ Jr. Fertil Steril 2002; 78: 491-9.
[29] WHO. Ann Ist Super Sanita 2001; 37: I-XII, 1-123.
[30] Berthier F, Lambert C, Genin C, Bienvenu J. Clin Chem Lab Med 1999; 37(5): 593-9.
[31] Wilson JD and Foster DW. Williams Text Book of Endocrinology. Elsevier, Philadelphia, 1999.
[32] Yesilli C, Mungan, G, Seçkiner I, Akduman B, Açikgöz S, Altan K, Mungan AG. Urology 2005; 66: 610-5.
[33] Handelsman DJ, and Cooper TG. Asian J Androl 2013; 15: 159-61.
[34] Brugo-Olmedo S, Chillik C, Kopelman S. Reprod Biomed Online 2001; 2: 41-53.

[35] Pacifici R, Altieri I, Gandini L, Lenzi A, Pichini S, Rosa M, Zuccaro P, Dondero F. Ther Drug Monit 1993; 15: 358-63.
[36] Kumosani TA, Elshal MF, Al-Jonaid AA, Abduljabar HS. Clin Biochem 2008; 41: 1199-203.
[37] Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. Mutat Res 1996; 351: 199-203.
[38] Feldman HA, Johannes CB, Derby CA, Kleinman KP, Mohr BA, Araujo AB, McKinlay JB. Prev Med 2000; 30: 328-38.
[39] Shiri R, Koskimäki J, Hakama M, Häkkinen J, Huhtala H, Tammela TL, Auvinen, A. Int J Impot Res 2004; 16: 389-94.
[40] Venners SA, Wang X, Chen C, Wang L, Chen D, Guang W, Huang A, Ryan L, O'Connor J, Lasley B, Overstreet J, Wilcox A, Xu XX. Am J Epidemiol 2004; 159: 993-1001
[41] Mlynarcikova A, Fickova M, Scsukova S. Endocr Regul 2005; 39: 21-32.
[42] Lam TH, Abdullah AS, Ho LM, Yip AW, Int J Impot Res 2006; 18: 364-9.
[43] Appasamy M, Muttukrishna S, Pizzey AR, Ozturk O, Groome NP, Serhal P, Jauniaux E. Reprod Biomed Online 2007; 14: 159-65.
[44] Hung PH, Baumber J, Meyers SA, VandeVoort CA. Reprod Toxicol 2007; 23: 499-506.
[45] Richthoff J, Elzanaty S, Rylander L, Hagmar L, Giwercman A. Int J Androl 2008; 31: 31-9.
[46] Calogero A, Polosa R, Perdichizzi A, Guarino F, La Vignera S, Scarfia A, Fratantonio E, Condorelli R, Bonanno O, Barone N, Burrello N, D'Agata R, Vicar, E. Reprod Biomed Online 2009; 19: 564-71.
[47] Hung PH, Froenicke L, Lin CY, Lyons LA, Miller MG, Pinkerton KE, VandeVoort CA. Reprod Toxicol 2009; 27: 140-8.
[48] Oyeyipo IP, Raji Y, Emikpe BO, Bolarinwa AF. Niger J Physiol Sci 2010; 25: 81-6.
[49] Al-Matubsi HY, Kanaan RA., Hamdan F, Salim M, Oriquat GA., Al Hanbali OA. Cent Eur J Public Health 2011; 19: 54-9
[50] Tawadrous GA, Aziz AA, Mostafa T. J Urol 2011; 186: 1986-90.
[51] Ghaffari MA and Rostami M. J Reprod Infertil 2012; 13: 81-7.
[52] Taha EA, Ez-Aldin AM, Sayed SK, Ghandour NM, Mostafa T. Urology 2012; 80: 822-5.
[53] Zhang ZH, Zhu HB, Li LL, Yu Y, Zhang HG, Liu RZ. Iran J Reprod Med 2013; 11: 589-96.
[54] Yousefniapasha Y, Jorsaraei G, Gholinezhadchari M, Mahjoub S, Hajiahmadi M, Farsi M. Cell J 2015; 17:129-36.
[55] Blanco-Munoz J, Lacasana M, Garduno CA. Sci Total Environ 2012; 426: 100-5.
[56] Trummer H, Habermann H, Haas J, Pummer KH. Hum Reprod 2002; 17: 1554-9.
[57] Lotti F, Corona G, Vitale P, Maseroli E, Rossi M, Fino MG, Maggi M. Hum Reprod 2015; 30: 590-602.
[58] Pasqualotto FF, Sobreiro BP, Hallak J, Pasqualotto EB, Lucon AM. BJU Int 2006; 97: 324-6.
[59] Meikle AW, Liu XH, Taylor GN, Stringham JD. Life Sci 1988; 43: 1845-50.
[60] Mendelson JH, Sholar MB, Goletiani N, Siegel AJ, Mello NK. Neuropsychopharmacol 2005; 30: 1751-63.