

Effect of Acetylcholinesterase and Butyrylcholinesterase on Intrauterine Insemination, Contribution to Inflammations, Oxidative Stress and Antioxidant Status; A Preliminary Report

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Abstract

Background: Oxidative stress affects women fertility and influences on the sperm quality by altering activities of cholinesterases, a molecular marker of stress-related infertility. The aim of the present study was to investigate the role of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) activities and phenotypes in patients with unexplained infertility (idiopathic). It's possible association with inflammation marker C-reactive protein (CRP) and other oxidative stress markers, *i.e.* before and after intra uterine insemination (IUI).

Methods: In this study, blood samples of 60 patients with unexplained infertility were collected the day before and 24 hr after IUI (between 8 AM and 9 AM after the overnight fasting) and activities of BuChE, AChE, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GpX) and serum levels of thiol proteins (TP), C-reactive protein (CRP), total antioxidant capacity (TAC) were measured. Statistical significance was assumed at $p < 0.05$.

Results: Before IUI, there was a significant ($p = 0.048$) positive correlation between BuChE activity and plasma TAC and a significant difference in the CAT activity between various BuChE (UU and non-UU) phenotypes. However, after IUI, a significant negative correlation between the AChE activity and BuChE activity was found ($p = 0.045$) and the level of RBC AChE activity was significantly reduced (382.4 ± 163.19 vs. 586.7 ± 384 IU/grHb, $p = 0.025$). Meanwhile, after IUI, the activities of SOD (1568 ± 847.5 IU/grHb vs. 1126 ± 229.3 , $p = 0.031$) and CAT (310 ± 53.4 IU/grHb vs. 338 ± 73 , $p = 0.025$) were increased.

Conclusion: This study suggests that decline in cholinesterases activities may be responsible for stimulation of oxidative stress and inflammation and reduction in fertility rates by IUI.

Keywords: Acetylcholinesterase (AChE), Butyrylcholinesterase (BuChE), Enzymatic and non-enzymatic antioxidant, Intrauterine insemination (IUI), Oxidative stress.

To cite this article: Vaisi-Raygani A, Keshvarzi F, Ferdowsi F, Goodarzi M, Rahimi Z, Baniamerian H. Effect of Acetylcholinesterase and Butyrylcholinesterase on Intrauterine Insemination, Contribution to Inflammations, Oxidative Stress and Antioxidant status; A Preliminary Report. *J Reprod Infertil.* 2016;17(3):157-162.

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Received: Aug. 1, 2015

Accepted: Nov. 16, 2015

Introduction

Infertility is relatively common among young couples (10-15%) where, 45-50% is due to female factor, 30-40% results from male fac-

tor, and the remaining of cases (10-30%) is idiopathic (1). One of the treatments of idiopathic infertility is ovulation induction followed by intra-

terine insemination (IUI) (2). Evidence suggests that oxidants such as active forms of oxygen are involved in occurrence of many destructive diseases and events including women fertility, menopause and fertility decline (3-5).

There is growing evidence on the effects of oxidative stress on the female genital tract, and also on infertility especially in men. In addition, the involvement of oxidative stress in the pathophysiology of preeclampsia, hydatidiform mole and birth defects has been reported (3-6).

Study in animal models has shown that acute inflammation is increased in intrauterine insemination compared to natural mating, resulting in neutrophils accumulation in the uterus (3-6).

Inflammation is part of the process of implantation and may be different from inflammatory process that is followed by IUI.

In addition, human and animal model studies have demonstrated that artificial insemination might change the rate of fertility due to activation of the cholinergic system that increases myometrial contractility. Several studies demonstrated that AChE inhibitors as agricultural insecticides were associated with yet unexplained impairments in spermatogenesis and sperm quality. Thus, maybe, the reduction of acetylcholine hydrolysis in uterus and the increase in uterus motility might have an effect on fertility in women (7-11). Studies have suggested that antioxidants may help in increasing the chances of fertilization.

There are two types of human cholinesterases, acetyl cholinesterase (AChE) and butyrylcholinesterase (BuChE) (12). AChE is found in the lungs, spleen, nerve endings, gray matter of the brain and red blood cells (13) and BuChE is found in plasma, and in different organs including liver, pancreas, heart, and brain (12). BuChE cleaves choline esters including the muscle relaxants, succinylcholine, mivacurium, and their derivatives, which are strong inhibitors of red cell acetylcholinesterase (14, 15).

Association of BuChE activity with oxidative stress, inflammatory diseases such as lupus, type 2 diabetes mellitus (T2DM), cardiovascular disease, rheumatoid arthritis, preeclampsia and with abnormal lipid profile during various phases of menstrual cycle in young healthy women with regular menses is well established (16-25). However, the physiological function of BuChE has not yet fully understood (12). The performance of AChE in the nervous system as well as the co-regulatory function of acetylcholine (ACh) in relation to BuChE

has been suggested (13). At present, no information on clinical significance of BuChE activity and phenotypes and its relationship with AChE activity, the marker of inflammatory (CRP), the enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and/or non-enzymatic (protein thiol and total antioxidant capacity) antioxidant defense system in IUI is available. The present study has investigated the association between BuChE activity and phenotypes with AChE and oxidative stress makers before and after IUI in patients with idiopathic infertility.

Methods

Subjects: The study protocol was approved by the Ethics Committee of the Kermanshah University of Medical Sciences (KUMS) and was in accordance with the principles of the Declaration of Helsinki II and all subjects provided written informed consent. The study population consisted of 60 (29.98±5.16 years old) patients with unexplained infertility that referred to the Infertility Center of the Kermanshah University of Medical Sciences for IUI.

In the present study, the kind of used catheter was Sage and easy-to-use. Sperm preparation technique that was used in this study was Discontinuous density gradient method according WHO laboratory manual (26).

Individuals with a history of illness, such as hypertension, chronic disease, myasthenia gravis, infectious diseases, cardiovascular, renal disease, diabetes, smoking and any diseases requiring medications were excluded from the study.

Chemical Analysis: The blood samples were collected before and 24 hr after IUI under the fasting condition. BuChE activity and phenotypes were determined using benzoylcholine chloride (50 $\mu\text{mol/l}$) as substrate in the presence or absence of dibucaine hydrochloride (10 $\mu\text{mol/l}$) and sodium fluoride (50 $\mu\text{mol/l}$) as inhibitor of enzyme described previously (25). One unit of BuChE activity was defined as the amount of enzyme required to hydrolyze 1 μmol of benzoylcholine chloride per minute at standard assay conditions. BuChE activity, DN and FN were calculated as follows:
 Serum BuChE (IU/L) = $\Delta A/\text{min} \times 30.3 \times 10^3$
 DN or FN = $(1 - \Delta A/\text{min with inhibitor} / \Delta A/\text{min without inhibitor}) \times 100$.

C-reactive protein (CRP) was measured using commercially available enzyme assay kits (Pars Azmon, Iran), using an automated Erba XL-600 (Mannheim, Germany).

Erythrocyte acetylcholinesterase activity was measured using acetylthiocholine as substrate as previously described (27). To obtain packed erythrocytes, an aliquot of blood samples containing heparin as anticoagulant was centrifuged at $500\times g$ for 15 min. The erythrocytes were washed three times with an isotonic solution of NaCl (0.9%) until a colorless supernatant was observed. To obtain erythrocyte hemolysate, 500 μ l of packed erythrocytes was hemolyzed by addition of four volumes of cold double distilled water. The resulting suspension was centrifuged twice to eliminate all of the cell membranes (28).

Erythrocyte SOD and GSH-Px activities and serum TAC levels were measured using commercially available kits (Randox Laboratories Ltd. Ireland). Erythrocyte CAT activity was measured by the method of Aebi et al. (29). Serum level of thiol proteins was measured using 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) as color reagent as previously described (30).

Statistical analysis: Plasma BuChE activity, levels of TP and TAC levels and activities of the erythrocyte AChE, Cu-Zn SOD, GSH-Px, and CAT (normal distribution using Kolmogorov-Smirnow) were compared before and after IUI using paired t-test. The correlation values of plasma TP and CRP, and the activities of the erythrocyte AChE, Cu-Zn SOD, GSH-Px, and CAT, with BuChE activity and phenotypes before and after IUI were calculated using Pearson correlation and an independent-samples t-test. An independent-samples t-test and Pearson correlation analysis were used to compare quantitative data. Statistical significance was assumed at the $p<0.05$. The SPSS statistical software package version 16 was used for the statistical analysis.

Results

The mean age of participants in the study was 29.98 ± 5.16 years and the mean body mass index (BMI) was 24.33 ± 2.64 Kg/m^2 . The activities of BuChE, AChE, Cu-Zn-SOD, GSH-Px, and CAT and the plasma level of CRP, TAC and thiol proteins before and after IUI in patients are given in table 1. The activity of AChE in RBC decreased significantly (586.7 ± 384.07 vs. 382.4 ± 163.19 $U/grHb$, $p=0.025$), while the activity of SOD (1126 ± 230 vs. 1568 ± 847.5 $U/grHb$, $p=0.031$) and CAT (310 ± 53.4 $U/grHb$ vs. 338 ± 73 , $p=0.025$) increased substantially (Table 1) following IUI.

A negative correlation between BuChE and AChE activity ($r=-0.44$, $p=0.045$) but a positive

Table 1. The activities and levels of parameters have been compared before and after IUI in patients

Parameters	Before IUI M \pm SD	After IUI M \pm SD	P-value
BuChE activity* (U/l)	1082 \pm 259	1033 \pm 248	0.31
AChE activity** (U/gHb)	586.7 \pm 384.1	382.4 \pm 163.2	0.025
SOD activity (U/gHb)	1126 \pm 230	1568 \pm 847.5	0.031
GSH-Px activity (U/gHb)	51.1 \pm 12.8	47.4 \pm 10.8	0.1
CAT activity (U/gHb)	310 \pm 53.4	338 \pm 73	0.025
TAC (mmol/l)	1.24 \pm 0.139	1.23 \pm 0.221	0.51
TP (μ mol/l)	761 \pm 165	745 \pm 164	0.61
CRP (mg/l)	1.5 \pm 0.78	1.83 \pm 1.23	0.09

* μ mol L^{-1} min^{-1} at 25°C, substrate benzoylcholine chloride; ** μ mol L^{-1} min^{-1} gHb at 25°C of pack cell, substrate acetylthiocholine; BuChE=butyrylcholinesterase; AChE=Acetylcholinesterase; CRP=C-reactive protein; SOD=superoxide dismutase; GpX=glutathione peroxidase; CAT=catalase (CAT); IUI=intrauterine insemination (IUI); TH=Thiol protein; Paired t test was used for comparing parameters before and after IUI

Table 2. Correlation between RBC AChE activity with activities and levels of parameters separately in patients before and after IUI

Parameters	RBC-AChE activity (U/grHb)	
	Before IUI	After IUI
BuChE activity (U/l)	$r=0.18$, $p=0.34$	$r=-0.44$, $p=0.045$
TAC (mmol/l)	$r=0.16$, $p=0.38$	$r=0.24$, $p=0.3$
TP (mmol/l)	$r=0.132$, $p=0.46$	$r=-0.029$, $p=0.9$
CAT (k/grHb)	$r=-0.07$, $p=0.7$	$r=-0.041$, $p=0.86$
GPX (U/grHb)	$r=-0.052$, $p=0.77$	$r=0.08$, $p=0.7$
SOD (U/grHb)	$r=-0.11$, $p=0.53$	$r=0.24$, $p=0.29$
CRP (mg/l)	$r=-0.12$, $p=0.4$	$r=-0.14$, $p=0.2$

r =Pearson Correlation; BuChE=butyrylcholinesterase; AChE= Acetylcholinesterase; CRP=C-reactive protein; SOD=superoxide dismutase; GpX=glutathione peroxidase; CAT=catalase (CAT); IUI=intrauterine insemination (IUI); TH=Thiol protein

Table 3. Correlation between serum BuChE activity with activities and levels of parameters separately in patients before and after IUI

Parameters	BuChE activity (U/l)	
	Before IUI	After IUI
AChE activity (U/gr Hb)	$r=0.18$, $p=0.34$	$r=-0.44$, $p=0.045$
TAC (mmol/l)	$r=0.27$, $p=0.048$	$r=-0.057$, $p=0.68$
TP (mmol/l)	$r=-0.017$, $p=0.9$	$r=-0.09$, $p=0.49$
CAT (k/grHb)	$r=-0.061$, $p=0.67$	$r=0.031$, $p=0.82$
GPX (U/grHb)	$r=-0.064$, $p=0.64$	$r=0.157$, $p=0.26$
SOD (U/grHb)	$r=-0.041$, $p=0.77$	$r=0.043$, $p=0.76$
CRP (mg/l)	$r=-0.11$, $p=0.42$	$r=-0.25$, $p=0.07$

r =Pearson Correlation; BuChE=butyrylcholinesterase; AChE=Acetylcholinesterase; CRP=C-reactive protein; SOD=superoxide dismutase; GpX=glutathione peroxidase; CAT=catalase (CAT); IUI=intrauterine insemination (IUI); TH=Thiol protein

correlation between BuChE and plasma level of TAC, following IUI ($r=0.027$, $p=0.048$) was found (Tables 2 and 3). In addition, carriers of non-UU

Table 4. Association of BuChE phenotypes with BuChE activity, activities and levels of parameters compared between UU and non-UU groups before and after IUI

Parameters	Before IUI			After IUI		
	UU	non-UU	P-value	UU	non-UU	P-value
AChE activity (<i>U/grHb</i>)	601.4±398.5	551±284.9	0.83	395±166.85	263±14.5	0.29
BuChE activity (<i>U/l</i>)	1083.7±257.6	1060±338.1	0.89	1040 ±240.4	906.7±395.8	0.87
TAC (<i>mmol/l</i>)	1.279±0.188	1.24±0.137	0.64	1.221±0.225	1.199±0.187	0.86
TP (<i>mmol/l</i>)	817.7±73.4	745±166.1	0.45	765.4±167.4	750±104.9	0.87
CAT (<i>k/grHb</i>)	308±49.2	372.2±98	0.041	336±73.8	375±72.6	0.078
GPX (<i>U/grHb</i>)	51.82±12.8	43.57±9.8	0.28	48.18±10.7	41±7.2	0.26
SOD (<i>U/grHb</i>)	1119±222	1134±357	0.91	1566±845.7	1577±1233	0.98
CRP (<i>mg/l</i>)	1.31±0.71	1.39±0.87	0.530	1.73±1.23	1.82±1.3	0.12

* Comparing parameters between BuChE phenotypes UU and non-UU by independent t test

BuChE phenotypes compared to UU carriers were found to have lower BuChE and AChE, and GSH-Px activities and lower levels of plasma TAC and thiol proteins regardless of IUI status. The non-UU BuChE carriers also had higher CAT and SOD activities than UU BuChE. However, regarding the CAT activity and CRP levels, the difference was not significant (Table 4).

Discussion

There are growing studies on the effect of acute inflammation and oxidative stress on female infertility (1, 5, 6, 9, 15). This study is unique in that it provides important information about possible stimulation of oxidative stress and acute inflammation by IUI that may lead to reduced fertility rates through an effect on the activities of AChE, BuChE and enzymatic and non-enzymatic antioxidant systems.

A significant decrease was found in activity of AChE in RBC following IUI. Reports of animal studies have shown that intramuscular injection of acetylcholine after natural mating increased uterine contraction and decreased fertility (4-6). It has also been demonstrated that the level of AChE in RBC significantly increased during pregnancy and returned to the normal pre-pregnancy level at 6th post-partum week (4-6, 31), suggesting that increased activity of AChE may inhibit the action of acetylcholine in the uterus to protect the pregnancy (Agarwal et al., 2012, de Peyster et al., 1994). These data collectively suggest that oxidative stress (oxidants) may help in decreasing the chances of fertilization.

In addition, BuChE activity decreased after IUI but it did not reach to a statistically significant level. However, there was a significant negative correlation between the BuChE activities with

AChE activity after IUI. It could be due to increased destruction of acetylcholine, reduced uterine contraction, and increased implantation of zygotes for a successful pregnancy. Recently, it was demonstrated that BuChE activity might be involved in the pathogenesis of preeclampsia through influence on lipid and lipoprotein metabolism and oxidative stress (22).

In this study, it was found that serum CRP concentration increased and plasma TAC and TP concentrations decreased following IUI. Some studies have shown that lower TAC in seminal fluid is associated with male infertility (32). That may cause oxidative damage to membranes and reduced sperm motility. Therefore, IUI may be the cause of oxidative stress in patients. There is growing evidence about the effects of oxidative stress on the female genital tract, and also infertility especially in men (1, 6, 33). These results are consistent with the results of previous studies (1, 6, 33, 34), demonstrating that oxidative stress (overproduction of ROS) may have an effect on female reproduction by disturbance of human oocyte intracellular Ca²⁺ homeostasis as well as oocyte maturation and fertilization. The bulk of evidence at the present time indicates that inflammation of the endometrium may reduce fertility and implantation of a fertilized ovum (35). Thus, the increase in CRP might be inversely related with the efficiency of IUI fertility. Tamura et al. demonstrated that the administration of melatonin resulted in reduced intra-follicular oxidative damage and a net elevation of fertilization and pregnancy rates (36).

Therefore, oxidative stress is involved in oocyte quality, and its degree can be evaluated by biomarkers of TAC, thiol proteins and lipid peroxidation (37). These findings suggest that reduced

antioxidant capacity of semen could be responsible for infertility.

The antioxidant and detoxification properties of BuChE directly correlate with its activity. Therefore, individuals with abnormal phenotypes of BuChE (non-UU phenotype with low BuChE activity) may be more susceptible to oxidative stress and may have a lower fertility rate when using IUI.

Conclusion

Results of this study demonstrated that IUI, due to increasing oxidative stress and decreasing cholinesterases activities, leads to increase in the action of acetylcholine on the uterus and may increase uterine contraction and motility and decrease fertility. However, because of the heterogeneous picture of IUI due to increased oxidative stress, acute inflammation and the influence of a subset of risk factors, in the treatments, IUI was used for infertile couples. Therefore, further studies with greater number of patients would be required to shed light on the contribution of non-UU phenotypes of BuChE and enzymatic activity such as BuChE, AChE, SOD, CAT and serum levels of TAC, TP and CRP for treatment of infertile couples in different ethnicities.

Acknowledgment

This work was performed in partial fulfillment of the requirements for MD degree of Dr Farivar Ferdowsi, Kermanshah University of Medical Sciences. This research was supported by a grant (No; 89070) from Kermanshah University of Medical Sciences.

Conflict of Interest

We have no conflict with anybody.

References

1. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril*. 2003;79(4):829-43.
2. Tanahatue SJ, McDonnell J, Goverde AJ, Hompes PG, Lambalk CB. Total fertilization failure and idiopathic subfertility. *Reprod Biol Endocrinol*. 2009; 7:3.
3. Lee VM, Wong JS, Loh SK, Leong NK. Sperm motility in the semen analysis affects the outcome of superovulation intrauterine insemination in the treatment of infertile Asian couples with male factor infertility. *BJOG*. 2002;109(2):115-20.
4. Mor I, Grisaru D, Titelbaum L, Evron T, Richler C, Wahrman J, et al. Modified testicular expression of stress-associated "readthrough" acetylcholinesterase predicts male infertility. *FASEB J*. 2001;15(11):2039-41.
5. Raynal P, Houdeau E. [Comparison of the uterine reflex activity during artificial insemination and mating in the ewe]. *J Gynecol Obstet Biol Reprod (Paris)*. 2004;33(8):725-33. French.
6. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol*. 2012;10:49.
7. Tielemans E, van Kooij R, te Velde ER, Burdorf A, Heederik D. Pesticide exposure and decreased fertilisation rates in vitro. *Lancet*. 1999;354(9177): 484-5.
8. Sarkar R, Mohanakumar KP, Chowdhury M. Effects of an organophosphate pesticide, quinalphos, on the hypothalamo-pituitary-gonadal axis in adult male rats. *J Reprod Fertil*. 2000;118(1):29-38.
9. Agarwal A, Said TM, Bedaiwy MA, Banerjee J, Alvarez JG. Oxidative stress in an assisted reproductive techniques setting. *Fertil Steril*. 2006;86(3):503-12.
10. Venkataraman BV, Iyer GY, Narayanan R, Joseph T. Erythrocyte and plasma cholinesterase activity in normal pregnancy. *Indian J Physiol Pharmacol*. 1990;34(1):26-8.
11. Vanderwall DK, Woods GL. Effect on fertility of uterine lavage performed immediately prior to insemination in mares. *J Am Vet Med Assoc*. 2003; 222(8):1108-10.
12. Kalow W. Human pharmacogenomics: the development of a science. *Hum Genomics*. 2004;1(5): 375-80.
13. La Du BN, Bartels CF, Nogueira CP, Arpagaus M, Lockridge O. Proposed nomenclature for human butyrylcholinesterase genetic variants identified by DNA sequencing. *Cell Mol Neurobiol*. 1991;11(1): 79-89.
14. Bartels CF, James K, La Du BN. DNA mutations associated with the human butyrylcholinesterase J-variant. *Am J Hum Genet*. 1992;50(5):1104-14.
15. Prabhu K, Kumar P, Adiga SK, Rao A, Lanka A, Singh J. Plasma protein thiols, ceruloplasmin, C-reactive protein and red blood cell acetylcholinesterase in patients undergoing intrauterine insemination. *J Hum Reprod Sci*. 2009;2(1):27-9.
16. Vaisi-Raygani A, Tavilani H, Vaisi-Raygani H, Rahimi Z, Karimi J, Pour-motabbed T. Serum butyrylcholinesterase activity and phenotype associations with Lipid profile during various phases of

- menstrual cycle in young healthy women with regular menses, a preliminary report. *AviJMB*. 2013;1(1):23-9.
17. Massoulie J, Pezzementi L, Bon S, Krejci E, Vallette FM. Molecular and cellular biology of cholinesterases. *Prog Neurobiol*. 1993;41(1):31-91.
 18. Vaisi-Raygani A, Rahimi Z, Entezami H, Kharrazi H, Bahrhemand F, Tavilani H, et al. Butyrylcholinesterase K variants increase the risk of coronary artery disease in the population of western Iran. *Scand J Clin Lab Invest*. 2008;68(2):123-9.
 19. Vaisi-Raygani A, Rahimi Z, Kharrazi H, Tavilani H, Aminiani M, Kiani A, et al. Determination of butyrylcholinesterase (BChE) phenotypes to predict the risk of prolonged apnea in persons receiving succinylcholine in the healthy population of western Iran. *Clin Biochem*. 2007;40(9-10):629-33.
 20. Vaisi-Raygani A, Tavilani H, Zahrai M, Rahimi Z, Sheikh N, Aminian M, et al. Serum butyrylcholinesterase activity and phenotype associations with lipid profile in stroke patients. *Clin Biochem*. 2009;42(3):210-4.
 21. Kalman J, Juhasz A, Rakonczay Z, Abraham G, Zana M, Boda K, et al. Increased serum butyrylcholinesterase activity in type IIb hyperlipidaemic patients. *Life Sci*. 2004;75(10):1195-204.
 22. Rahimi Z, Ahmadi R, Vaisi-Raygani A, Rahimi Z, Bahrhemand F, Parsian A. Butyrylcholinesterase (BChE) activity is associated with the risk of pre-eclampsia: influence on lipid and lipoprotein metabolism and oxidative stress. *J Matern Fetal Neonatal Med*. 2013;26(16):1590-4.
 23. Iwasaki T, Yoneda M, Nakajima A, Terauchi Y. Serum butyrylcholinesterase is strongly associated with adiposity, the serum lipid profile and insulin resistance. *Intern Med*. 2007;46(19):1633-9.
 24. Bahrhemand F, Vaisi-Raygani A, Rahimi Z, Ahmadi R, Kiani A, Tavilani H, et al. Synergistic effects of BuChE non-UU phenotype and paraoxonase (PON1) 55 M allele on the risk of systemic lupus erythematosus: influence on lipid and lipoprotein metabolism and oxidative stress, preliminary report. *Lupus*. 2014;23(3):263-72.
 25. Shahmohamadnejad S, Vaisi-Raygani A, Shakiba Y, Kiani A, Rahimi Z, Bahrhemand F, et al. Association between butyrylcholinesterase activity and phenotypes, paraoxonase192 rs662 gene polymorphism and their enzymatic activity with severity of rheumatoid arthritis: correlation with systemic inflammatory markers and oxidative stress, preliminary report. *Clin Biochem*. 2015;48(1-2):63-9.
 26. WHO. WHO laboratory manual for the examination and processing of human semen. 5th ed. Switzerland: WHO press; 2010. 286 p.
 27. Whittaker M, editor. Cholinesterase. Vol. 11, Monograph in Human Genetics. Switzerland: Karger; 1986. 31 p.
 28. Marcus DL, Thomas C, Rodriguez C, Simberkoff K, Tsai JS, Strafaci JA, et al. Increased peroxidation and reduced antioxidant enzyme activity in Alzheimer's disease. *Exp Neurol*. 1998;150(1):40-4.
 29. Aebi H. Catalase in vitro. *Methods Enzymol*. 1984;105:121-6.
 30. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959;82(1):70-7.
 31. de Peyster A, Willis WO, Liebhaber M. Cholinesterase activity in pregnant women and newborns. *J Toxicol Clin Toxicol*. 1994;32(6):683-96.
 32. Pedron N. Cholinesterase activity of seminal plasma and human spermatozoa in normal and infertile subjects. *Arch Androl*. 1983;10(3):249-51.
 33. Tham E, Liu J, Innis S, Thompson D, Gaylinn BD, Bogarin R, et al. Acylated ghrelin concentrations are markedly decreased during pregnancy in mothers with and without gestational diabetes: relationship with cholinesterase. *Am J Physiol Endocrinol Metab*. 2009;296(5):E1093-100.
 34. Omu AE, Al-Azemi MK, Omu FE, Fatinikun T, Abraham S, George S, et al. Butyrylcholinesterase activity in women with diabetes mellitus in pregnancy: correlation with antioxidant activity. *J Obstet Gynaecol*. 2010;30(2):122-6.
 35. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl*. 2003;49(2):83-94.
 36. Gavella M, Lipovac V, Vucic M, Rocic B. Relationship of sperm superoxide dismutase-like activity with other sperm-specific enzymes and experimentally induced lipid peroxidation in infertile men. *Andrologia*. 1996;28(4):223-9.
 37. Miesel R, Jedrzejczak P, Sanocka D, Kurpisz MK. Severe antioxidant deficiency in human semen samples with pathological spermogram parameters. *Andrologia*. 1997;29(2):77-83.