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Original Research Article

Effect of L-Carnitine and Glutathione Supplemented to FertiCult Flushing Medium on Human Sperm Parameters During *in vitro* Sperm Activation in Asthenozoospermic Men

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Abstract

L-Carnitine (LC) plays important role in sperm metabolism, act as antioxidant and provides readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and spermatogenic process. Glutathione (GSH) has several biological functions found widely in animal body, in somatic cells and also in gametes. It has an important role in anti-oxidation process of endogenous and exogenous composts. The **aim of this study was designed to investigate the effect of combination of LC and GSH on human sperm function parameters of Asthenozoospermic men. The study included forty five of asthenozoospermic infertile men as study group and twenty five normozoospermic fertile men as control group. Ejaculated semen were obtained from patients and divided into two equal portions, first portion was activated by free FertiCult Flushing medium as (control), and second portion activated by L-Carnitine and Glutathione (GSH) in 5mM concentration to each one, prepared sperm by centrifugation –swim up technique, and examined microscopic parameters. Result of *in vitro* sperm activation by LC and GSH was significant (P<0.05) decrease in sperm concentration, highly significant increase (P<0.001) in sperm motility, and highly significant decrease in immotile sperm (grade D) and significant increase (P<0.05) in morphologically normal sperm (MNS) compared with pre-activation and control medium. This study has been shown a positive effect and improvement in certain sperm functional parameters after *in vitro* sperm activation by LC and GSH supplemented medium.**

Keywords: L-Carnitine, Glutathione, FertiCult Flushing Medium, Sperm Function Parameters, *in vitro* sperm activation

INTRODUCTION

Male infertility has been commonly defined as the inability to conceive after 12 months regular intercourses, with the absence of contraceptives and the wife didn't appear any clear reason for not getting pregnancy (Gnoth et al., 2005). Male barrenness is a multifaceted illness process with various potential contributing causes. The larger part of male infertility causes are because of inadequate sperm generation of obscure starting point, ecological and wholesome factors (Sinclair, 2000). Some men are infertile because of poor sperm motility, this condition is called Asthenospermia or Asthenozoospermia (astheno = weak) (World Health Organization, 2010). Reactive oxygen species (ROS) have a central role in normal sperm functions and at low levels are involved with capacitation, acrosome reaction, stimulating hyper-activation and binding to zona pellucida of oocyte (Ford, 2004). Aerobic metabolism is associated with the generation of ROS (Agarwal et al., 2004b). It appear elevated in sperm samples as a result of cellular necrosis, morphological errors and following cryopreser-

vation, and because of spermatozoa have high content of poly unsaturated fatty acids, become highly susceptible to free radicals and ROS (Cocchia et al., 2011). Antioxidant can be described as a substance, which can dramatically delay or prevent oxidation of an oxidizable substrate (Sajal et al., 2009). Antioxidants were classified into enzymatic antioxidants, which are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX), and non-enzymatic antioxidant such as vitamin C, vitamin E, Pyruvate, Glutathione, and Carnitine (Agarwal and Saleh, 2002). L-Carnitine is an amino corrosive subordinate with the name β-hydroxy-γ-trimethyl-aminobutyrate, it plays a key role in energy provision in cells of humans and animals, as a co-factor (Baumgartner and Jacobs, 1999). L-Carnitine transports long-chain fatty acids across the mitochondrial membrane for β-oxidation to generate adenosine triphosphate (ATP) energy, and aids in release of stored body fat, and triglycerides into blood stream for energy (Uhlenbruck, 1996). It plays critical role in maturation and motility of spermatozoa within the male reproductive tract (Ng et al., 2004). Glutathione (L-glutamyl-L-cysteinyl-glycine; GSH). It is thiol tripeptide, composed of glutamate, cysteine, and glycine. The cysteine subunit provides an exposed free sulfhydryl group (SH) that directly scavenges against damage and free radicals and GSH plays indirect roles cofactor for glutathione peroxidase, which uses glutathione to reduce hydrogen peroxide to H2O and lipoperoxides to alkyl alcohols (Bilodeau et al., 2000). Cellular GSH play a key role in many biological processes, including the synthesis of proteins and DNA, and the transport of amino acids (Naher et al., 2011).

MATERIALS AND METHODS

The study was conducted in High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. The study included forty five asthenozoospermic subjects and twenty five normozoospermic subjects. Semen samples were collected by masturbation into wide-mouth containers, after 3-7 days of sexual abstinence, placed in an incubator at 37°C till complete liquefaction. Semen samples were analyzed by a macroscopic and microscopic examination according to standard criteria of WHO 1999 and 2010.

Macroscopic Examination

Appearance, Volume, Liquefaction time, Viscosity, pH

Semen sample were examined within one hour of ejaculation at room temperature. The semen is considered normal when the appearance is gray. Volume of the ejaculate was measured using a graduated cylinder with a conical base. Normal semen sample liquefies within 60 minutes at room temperature, although this usually occurs in a period less than this time. Viscosity of the semen sample was estimated by a gentle aspiration into Pasteur pipette. The sample is considered normal when the semen leaves the pipette as small drop by drop. The pH of the semen was measured by using pH litmus paper. The pH of the semen is considered normal when it is slightly alkaline and ranges between (7.2-8.0) according to criteria 2010.

Microscopic Examination

Microscopic examination was performed (1) Before activation (fresh). (2) After activation, by FertiCult Flushing medium only (Control). (3) After activation by combination of (LC and GSH). A drop (10µl) of sperm sample was placed on warm slide and covered with cover slip and examined under light microscope, X40 objective lens. Results were reported according to 1999 and 2010 of WHO manual.

Sperm Concentration

It was measured from the mean number of sperm in five high power fields (HPF) under magnification of X 40 objective lens. This number was multiplied by a factor of one million.

Sperm Motility

Number of motile sperm in five randomly selected fields away from the cover slip edge was counted. At least one hundred spermatozoa were counted, and the number of progressively motile and immotile sperm was documented, then sperm were counted in four categories (WHO, 1999).

Assessment of Sperm Morphology

Examination of morphologically normal sperm was performed by Hematoxylin-Eosin stain that was evaluated by placing 5µl of Semen samples on slide, thin smear made, and dried in air at room temperature, fixed and stained by Hematoxylin-Eosin stain. At least 200 sperm were scored on randomly chosen fields, under oil immersion with high resolution (X100) objective lens, the assessment criteria can be illustrated in figure(1).

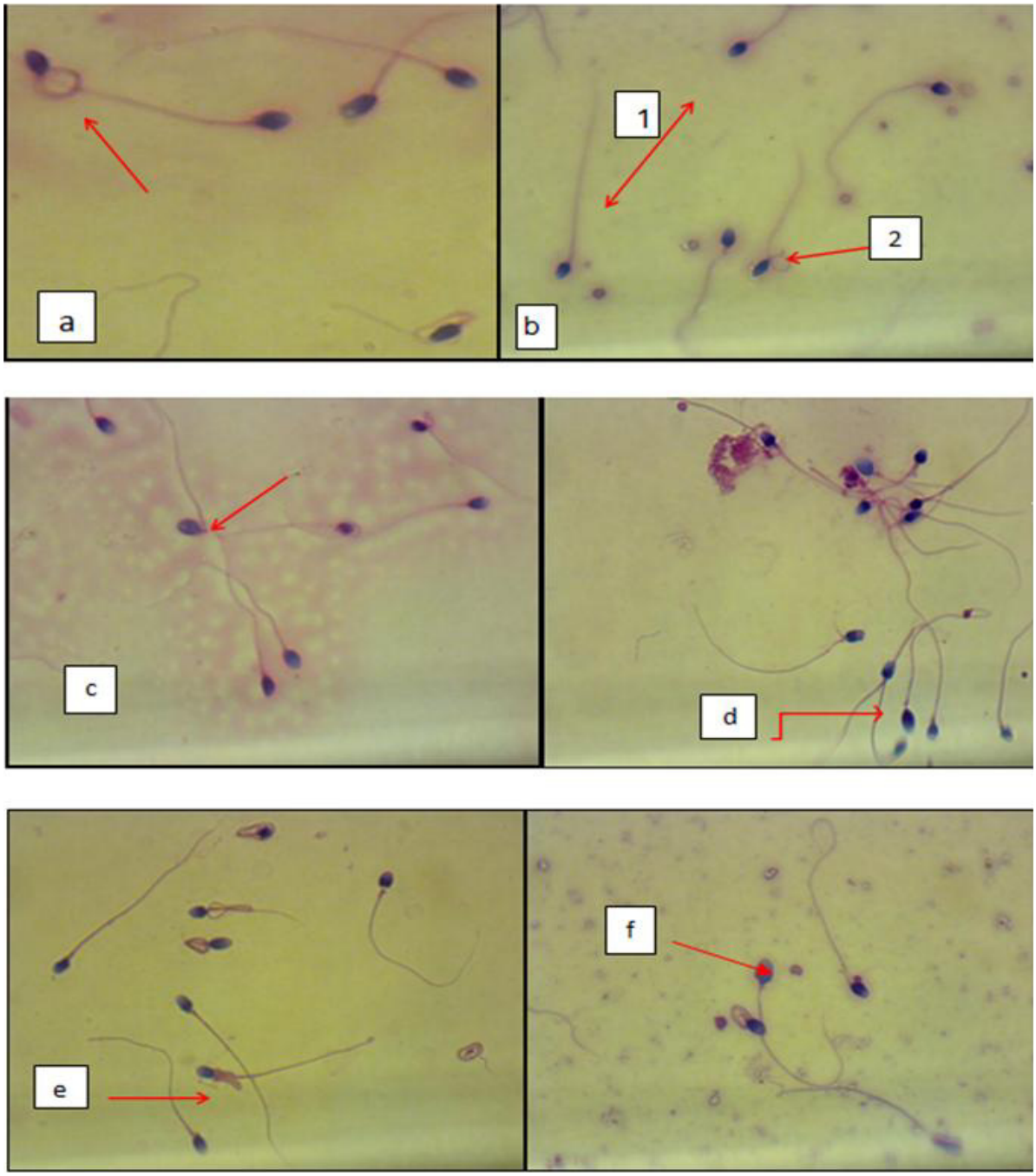

Figure 1. Sperm stained with Hematoxylin-Eosin (100X):
a- Abnormal, coiled tail, b- 1-normal, 2-abnormal pyriform head,
c-Abnormal, amorphous head with broken neck, d- Abnormal double tail,
e- Abnormal, cytoplasmic droplet,f- Abnormal, large head.

Preparation for *in vitro* sperm activation

L-Carnitine + Glutathione Solution

It was prepared in concentration 5mM of LC and 5 mM of Glutathione by adding 0.8 mg of LC and 15.4 mg of GSH to 10 ml of FertiCult Flushing medium. After well shaking it was filtered by using millipore 0.45µM and had been fixed for 7.4-7.8 PH at room temperature, and sterilized in U\V light.

Centrfiugation Technique (simple wash and swim up)

After liquefacation of ejaculated samples, one volume of semen was placed in a conical test tube and diluted with

one volume of culture medium and gently inverted twice to mix the components. Centrfiugation at 2500 round per minute (rpm) for 5 minutes. Supernatant was removed by pasteur pipette to obtain the pellet, and about 0.5-1 ml of media was added to the final sperm pellet. Examination was done after 30 minutes under light microscope by taking 10µl of simple from the top and the middle of the tube.

Statistical Analysis

The Statistical Analysis System-SAS (2016) program was used to measure the effect of different factors in study parameters. Least significant difference-LSD test (ANOVA) was used to compare between means (Statistical Analysis System, 2016). Differences between values were considered significant at P<0.05 and high significant at P<0.001.

RESULTS

Effect of *in vitro* activation by Free FertiCult medium (control) on certain sperm function parameters of asthenozoospermic men and normozoospermic men using centrifugation-swim up technique

In study group (Asthenozoospermic men), mean sperm concentration (million/ml) after *in vitro* activation using FertiCult medium only (control), was highly significant decrease (P<0.001) compared to the result before activation. There was no significance in active sperm motility grade A, there was significant increase (p<0.05) in grade B, grade A+B and Grade C compared to semen before activation. There was high significant (p<0.001) decrease in immotile sperm grade D and significant (P<0.05) increment in percentage of morphologically normal sperm (MNS) following activation with FertiCult medium as shown in table (1).

On the other hand, in Normozoospermic male (control group), FertiCult media resulted in the following: highly significant reduction in sperm concentration, significant increase in grade A, grade B, grade A+B, and grade C sperm %, highly significant reduction in grade D sperm % and significant increase in MNS % as shown in table (2).

Effect of *in vitro* activation by medium containing L-Carnitine (LC) combined with Glutathione (GSH) on certain sperm function parameters of Asthenozoospermic men and Normozoospermic men using centrifugation-swim up technique

The mean sperm concentration (million/ml) after *in vitro* activation using medium supplemented with LC+GSH was significantly decreased (P<0.05), compared to result before activation and significant difference compared with control medium. There was significant (P<0.05) increase in active sperm motility grade A. Highly significant (P<0.001) increment in active sperm motility grade B and grad A+B. Grade C was non-significantly deferent when activated by LC+GSH compared with fresh semen. There was highly significant (P<0.001) decrease in the immotile sperm (grade D) and significant (P<0.05) increase in percentage of MNS compared results before activation (fresh) and control medium as shown in table(1).

Same results was obtained from control group (Normozoospermia), there was significant reduction in sperm concentration, highly significant increase in grade A sperm %, grade B sperm %, and grade A+B sperm %. There was no significance in grade C sperm %, highly significant reduction in grade D sperm % and significant increase in MNS compared results before activation (fresh) and control medium as shown in table(2).

DISCUSSION

Effect of *in vitro* activation by Free FertiCult medium (control) on certain sperm function parameters

There was highly significant P<0.001 reduction in concentration of spermatozoa following *in vitro* activation by free FertiCult medium due to the inability ofthe dead and abnormal sperm to swim up and migrate from sperm pellet to the higher layer of culture medium. These results were in agreement with other studies using culture medium for the activation of sperm *in vitro* (Allaw, 1999).

Concerning sperm motility, there was a significant increase in sperm motility compared with pre activation. These cultures contain many ions such as sodium, potassium, calcium, magnesium, phosphate, pyruvate and lactate. These ions work as source of energy to activate sperm that increases their movement (Al-Obidy, 2010). This was in agreement with study of (Yahya et al., 2013). There was a significant decrease in non-motile sperm (grade D may be refer to that only motile sperm swim up to the higher layer while dead and immotile sperm remain in the lower of the medium, and this was consistent with the findings of the (Makler and Shiran, 1998). Our study found significant (P<0.05) increment in morphologically normal sperm (MNS) compared with results before activation. It may be because of sperm with normal shapes considered by their greater activity while sperm with abnormal shapes, which characterized by weak motility remain in the bottom (Al-Shimerty, 2014).

Effect of *in vitro* activation by Free FertiCult medium supplemented with L-Carnitine and Glutathione on certain sperm function parameters

There was no previous studies reported activation of poor

Table 1. Certain sperm function parameters after *in vitro* activation by free FertiCult control medium and LC combination with GSH supplemented medium of asthenozoospermic men using centrifugation-swim up technique

Certain sperm function Parameters		Before activation (Fresh Semen) (M ±S.E)	After Activation By FertiCult Medium only (control) (M±S.E)	After Activation by LC+GSH Supplemented Medium (M±S.E)
Sperm concentration (Million/ml)		52.73± 1.89a	23.48±0.91c	32.44 ±1.0 b
Progressive sperm motility (%)	**Grade A**	0.22± 0.15c	2.75±0.43c	14.28±0.90 a
	Grade B	33.28± 1.08 c	40.00 ± 0.88 bc	51.55 ±1.05 a
	Grade A+B	33.51± 1.14b	42.75 ± 1.14c	65.84 ±1.48 a
Non progressive sperm motility (%)	**Grade C**	28.77±1.12c	39.66 ± 1.25 a	30.62 ±1.15 bc
Immotile sperm (%)	**Grade D**	37.82± 1.39a	17.35 ± 1.06b	3.35 ±0.71 c
Morphologically Normal Sperm (%)		30.06 ± 1.39 c	35.00 ± 0.50 bc	42.95 ±0.56 a

M±SE = Mean ± Standard Error Different letters mean a significant
Same letters mean non-significant
Number of asthenozoospermic Men =45

Table 2. Certain sperm function parameters after in vitro activation by free FertiCult medium (control) and LC combination with GSH supplemented medium of normozoospermic men using centrifugation-swim up technique

Certain sperm function Parameters		Before activation (Fresh Semen) (M ±S.E)	After Activation By FertiCult Medium only(control) (M±S.E)	After Activation by LC+GSH Supplemented Medium (M±S.E)
Sperm concentration (Million/ml)		73.48± 1.80 a	33.80 ± 1.01 c	42.40±0.82 b
Progressive sperm motility (%)	**Grade A**	9.32± 0.85 d	14.52 ± 1.02 cd	27.32 ±1.11 a
	Grade B	46.20± 0.92 b	50.80 ± 0.98 ab	54.60 ±1.20 a
	Grade A+B	55.52 ± 0.90 c	65.32 ± 1.23 b	81.92±1.33 a
Non progressive sperm motility (%)	**Grade C**	20.96 ± 0.89 b	31.28 ± 1.26 a	18.08 ±1.33 b
Immotile sperm (%)	**Grade D**	22.68± 0.90 a	3.40 ± 0.74 b	0.00±0.00 b
Morphologically Normal Sperm (%)		35.12 ± 0.84 c	39.60 ± 0.70 bc	47.80 ±0.70 a

M±SE = Mean ± Standard Error Different letters mean a significant
Same letters mean non-significant
Number of normozoospermic Men =25

sperm by LC combination with GSH. Our study showed significant differences in sperm concentration compared with control medium, also showed high significant increment in sperm motility (grade A, grade B, and A+B). This findings may resulted from the complementary effect of LC and GSH each other on the cycle of energy supplementation and enhancement of sperm motility and concentration, because more motile sperm can move and swim up and migrate from immotile sperm and pellet to the upper layer of culture medium (Jeulin C. and Lewin L.). It had been shown that LC can increase ejaculatory sperm motility of men with Asthenozoospermia (Jeulin and Lewin, 1996). In another study, (Shi, *et al*.) demonstrated that testicular sperm motility improved after exposure to LC *in vitro* activation (Shi et al., 2010). L-Carnitine increases sperm motility by assisting energy production due to carrying fatty acids from sperm mitochondria (Agarwal and Said, 2004). Similarly, GSH has been reported to increase sperm motility, these findings were compatible with that observed by (Ismail, *et al*.) who studied on Egyptian Buffalo Semen and noted that individual motility, viability, intact acrosome and plasma membranes of sperm were significantly improved by inclusion of GSH in the medium (Ismail et al., 2011) . Other researchers have reported that ROS by interruption of adenosine triphosphate (ATP) production or flagellar axonome phosphorylation can damage sperm motility (Gil-Guzman et al., 2001). Oxidative stress can influence cell membrane structures, therefore, ROS scavengers such as LC and GSH may protect plasma membrane, reduce the damage and improve sperm motility and morphology (Jenkins and Griffith, 1986).

CONCLUSIONS

By the findings of the present study, it was concluded that:

1- Morphologically normal sperm and progressive sperm motility were highly significant increased after *in vitro* activation by media supplemented with LC+GSH compared with pre-activation and control medium, using centrifugation –swim up technique.

2- Sperm immotility was highly significant decreased after activation by LC+GSH media compared with pre-activation and control medium.
3- Sperm concentration was improved after *in vitro* activation with media containing LC+GSH compared to control media.

REFERENCES

Agarwal A, and Saleh RA (2002). Role of oxidants in male infertility: rationale, significance, and treatment. *UrolClin North Am*. 29: 817-827.

Agarwal A, Nallella KP, Allamaneni SS, and Said, TM (2004b). Role of antioxidants in treatment of male infertility: an overview of the literature. *Hum. Reprod.* 8: 616–627.

Agarwal A, Said TM (2004). Carnitine and male infertiity.*Reprod. Biomed*; 8: 376 384.

Allaw AK (1999). Treatment and *In Vitro* Sperm Activation for Immunologically Infertile Patients M.Sc. Thesis, College of Medicine,Kufa University.

Al-Obidy S (2010). Effect of using the FertiCult Flushing medium in sperm parameters activation *in vitro* infertile patients suffering from Ashthenospermia. M.Sc. thesis, College of Sciences, Kufa University.

Al-Shimerty DF (2014). In-vitro Human Sperm Activation of Infertile Patients Suffering from Oligozoospermic by using Different Techniques. M.Sc. thesis, College of Sciences, Kufa University.

Baumgartner M, Dr. Jacobs ST (1999). L-Carnitine: Importance For Pig Breeding.; 22 , page 15.

Bilodeau JF, Chatterjee S, Sirard MA, and Gagnon C (2000). Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. *Mol. Reprod. Dev*. 55: 282-288.

Cocchia N, Pasolini MP, Mancini R, Petrazzuolo O, Cristofaro I, Rosapane I (2011). Effect of SOD (superoxide dismutase) protein supplementation in semen extenders on motility, viability, acrosome status and ERK (extracelular signal-regulated kinase) protein phosphorylation of chilled stallion spermatozoa. *Theriogenology*; 75:1201.

Ford WL (2004). Regulation of Sperm Function by Reactive Oxygen Species. *Human Reproduction Update*. 10 (5): 387-399.

Gil-Guzman E, Ollero M and Lopez MC *et al.* (2001). Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation.*Hum Reprod.* 16(9):1922-30.

Gnoth C, Godehardt E, Frank–Herrmann P (2005). Definition and prevalence of subfertility. *Hum. Reporд*; 1144-1147.

Ismail I, El-kon, Darwish Samy A (2011). Effect of Glutathione (GSH) on Microscopic Parameters and DNA Integrity in Egyptian Buffalo Semen During Liquid and Frozen Storage, *J. Reprod. Infertility*; 2 (3): 32-40,

Jenkins DL, Griffith OW (1986). Antiketogenic and hypoglycemic effects of aminocarnitine and acylaminocarnitines.*ProcNatlAcadSci* U S A. 83(2): 290-4.

Jeulin C, Lewin LM (1996). Role of free L-Carnitine and acetyl L-Carnitine in post-gonadal maturation of mammalian spermatozoa.*Hum Repord.* 2(2): 87-102.

Makler AStoller J, and Shiran EM (1998). Dynamic aspects concerned with mechanism of separating motile sperm from non-motile sperm, leukocytes, and debris with the use of high – density percoll. Gradients.*Fertil.Steril*.; 70:961 – 966.

Naher ZU, Biswas SK, Mollah FH, Ali M, Arslan MI (2011). Role of Glutathione in Male Infertility, Bangladesh . *J Med. Biochem*. 4(2): 20-25.

Ng CM, Blackman MR, Wang C (2004). The role of Carnitine in the male reproductive system. In: Carnitine: The Science behind a Conditionally Essential Nutrient. Eds: Alesci S, Manoli SI, Costello R,*et al*. New York. Vol. 1033: 177-188.

Sajal G, Neena M, Dipika S, Anjali C, Agarwal A (2009). Oxidative stress and it is role in female infertility and assisted reproduction: Clinical Implication. *Int. J. Fertil.Steril*.; 2:147-164.

Shi JZ, Zhang SS, Zhang Z, Liang Q, Shi Y, Hua JL (2010). Expressions of sperm-specific genes in Carnitine-cultured testis sperm of obstructive azoospermia patients.Chinese.*Zhonghua Nan KeXue*. 16(6): 504 -9.

Sinclair S (2000). Male infertility: nutritional and environmental considerations. *Alternat Med*. 5(1):28-38.

Statistical Analysis System, User's Guide (2016). Statistical. SAS. Version 9.1th ed. SAS.Inst. Inc. Cary.N.C. USA.

Uhlenbruck G (1996). L-Carnitine and the immune system: from the mode of metabolism to the modulation of membranes. In: Carnitine - Pathochemical Basics and Clinical Applications. Seim H, Löster H (Eds), Ponte Press Bochum. 47-60.

World Health Organization (1999). WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 4th ed. Camb Univ. Press, Cambridge, UK:128.

World Health Organization (2010). WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 5th ed. Camb Univ. Press, Cambridge, UK.

Yahya K, Al-Sultani S R, Al-Katib S (2013). Effect of vitamin C on *in vitro* sperm activation of asthenozoospermic infertile patients.*Ame. J. Res. Comm.*; 1(10): 40-48.