In vitro treatment of Ham's F-10 medium supplemented with vitamin C and E on human semen characteristic in asthenozoospermic men

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Abstract

Background: The levels of reactive oxygen species are normally limited by antioxidant defense mechanisms such as vitamin C and E that are present within seminal plasma and sperm plasma membrane. The Supplementing infertile males with antioxidant vitamin C and E is suggested as a potential treatment for idiopathic male infertility.

Objective: This study was designed to determine the effect of Ham's F-10 preparation medium supplemented with antioxidant vitamin C or E on semen samples prepared by conventional layering technique**.**

Methods: Liquefied semen (1ml) was layered beneath Ham's F-10 (1ml) enriched with 0.75 mg/ml vitamin C or E after in vitro sperm processing. However, semen samples were collected from a total of 60 asthenozoospermic men by masturbation after 3-5 days abstinence and allowed to liquefy at 37ºC in 5% CO_2 for 30 minutes and evaluated according to standard world health organization (WHO) criteria before and after in vitro sperm activation. The semen samples were divided into three groups, one group considered as a control group which had no antioxidant added, and the other two groups were prepared in the presence of antioxidant treatment (either vitamin C or vitamin E).

Results: The supplementation of sperm preparation medium with vitamin C or vitamin E significantly (P<0.001) improved and augmented the seminal parameters including sperm concentration, sperm motility, progressive sperm motility, and normal sperm morphology when compared to that of the control group.

Conclusion: It was concluded that supplementation of medium with antioxidant vitamin C or E actually improve sperm quality, but the better improvement appeared to be with vitamin C.

Key words: Antioxidant, vitamin C, sperm preparation technique, asthenozoospermia

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Introduction

Infertility affects 15% of couples and is cause of infertility in 30% of those couples is associated with aberrations found in male partner termed male infertility. Many cases of male infertility were previously considered idiopathic but are now being attributed to oxidative sperm damage resulting from the pathologically increased levels of reactive oxygen species [(1)].

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In contrast, high levels of ROS are harmful and lead to lipid peroxidation However, ROS can be produced by immature spermatozoa and leukocytes [(2)]. In normal sperm physiology, low levels of ROS are beneficial to stimulate sperm capacitation, enhance zona pellucida binding and promote acrosome reaction [(3).]

of sperm plasma membrane and DNA fragmentation [(4)]. Increased lipid peroxidation is associated with impaired sperm motility and diminished capacity for sperm-oocyte fusion [(5)]. One study found that men with high levels of ROS were 7 times less likely to achieve a pregnancy than men with low levels [(6)].

The levels of ROS are normally limited by antioxidant defense mechanisms such as vitamin C and E that are present within the seminal plasma and sperm plasma membrane [7]. However, the supplementing infertile males with antioxidant vitamin C and E suggested as a potential treatment for idiopathic male infertility [8] .Vitamin E is a chain-breaking antioxidant because of its ability to terminate a free radical chain reaction and play an important role in pathogenesis of male infertility and protecting against oxidative attack both in vivo and in vitro [9]. Specifically, vitamin E inhibits peroxidation of polyunsaturated fatty acids (PUFA) which is especially important in spermatozoa due to their high PUFA content [10]. While, Vitamin C actually secreted from seminal vesicles during ejaculation and protect human sperm from endogenous oxidative DNA damage [11]. It is acts as a scavenger of a wide range of ROS which explains its ability to successfully counteract the effects of DNA damage and ROS production [12]. It has previously been shown to be the major antioxidant in seminal plasma of fertile men contributing up to 65% of the total chain-breaking antioxidant capacity [13]. In addition, concentration of Vitamin C in seminal plasma is 10 times greater than the concentration found in blood plasma [14]. The semen quality is an important factor in determining suitability of the couples in achieving pregnancy. The fertilization occurs despite an abnormal semen analysis, or it fails to occur when analysis values are normal. The semen analysis cannot ascertain the functional capacity of sperm and frequently fails to predict the outcome of male infertility [15].

Subjects, Materials and Methods

Collection of semen samples:

Semen samples were obtained from a total of 60 asthenozoospermic men and attendance of IVF Institute of Embryo Research and Infertility Treatment/Al-Nahrain University between March and May 2007. The mean of age ± S.E.M for infertile subjects was 30.05 ± 4.87 years. The ejaculates were collected by masturbation after 3-5 days abstinence and allow liquefying at 37ºC in 5% CO_2 for 30 minutes. The liquefied semen is carefully mixed for few seconds, and then seminal fluid analysis parameters including sperm concentration, sperm motility, progressive sperm motility, and normal sperm morphology was examined before and after in vitro sperm treatment. However, WHO (1999) criteria for normal semen values were applied.

Sperm preparation technique and in vitro antioxidant treatment:

Sperm processing prepared using conventional layering technique by mixing 1ml of the liquefied semen was layered beneath 1ml culture medium (Ham's F-10) after finished the routine semen analysis and confirmed the results before in vitro sperm preparation which is regarded as a control. The supernatant was removed and divided into two tubes, 0.5 ml for each tube. One tube was mixed with 0.75 mg/ml antioxidant vitamin C (Sigma Aldrich Co. Ltd, Poole, UK) and another tube was mixed with 0.75 mg/ml antioxidant vitamin E (Trolox, Sigma Aldrich, UK).

Statistical analysis

Statistical analysis was performed with the SPSS version 12.00. The data analysis was done using paired sample t-test to assess the statistical differences in the results . Mean and standard error of mean (S.E.M) obtained from crude data to

compare between Pre-and Post-activation for semen parameters. P-value < 0.05 was used as a level of statistically significance.

Results

The results of the present study showed that semen samples supplemented with antioxidant vitamin C and E improved the results for semen parameter as compared with control group used Ham's F-10 only (Tables 1-3; respectively), but vitamin C gives the best seminal parameter including sperm concentration, sperm motility, progressive sperm motility, and normal sperm morphology as compared with vitamin E. However, it was noticed a significant (P<0.001) differences in sperm function and seminal fluid parameters were assessed post in vitro sperm activation as compared with results of pre-activation of human spermatozoa in all infertile patients.

The markedly reduction in sperm concentration was observed following in vitro sperm preparation using Ham's F-10 medium with and without vitamin C and vitamin E supplementation. The handling yielded significantly lower sperm concentration as compared with pre-activation. But, these parameters of spermatozoa significantly increased not only by addition of antioxidant within culture medium for sperm preparation as compared with unprepared semen, but also in absence of antioxidant within Ham's F-10 medium. It was recognized that Ham's F-10 medium contains protein, inorganic ions as well as carbohydrates, and most necessary requirement for improvement sperm functions that cause an increase in migration of normal mature active sperm to upper layer of culture medium.

Table 1: Effect of Ham's F-10 medium on semen parameters pre and post in vitro sperm activation for infertile patients* with asthenozoospermia

Parameters	Conventional layering technique	
	Pre-activation	Post-activation
Sperm Concentration ($\times 10^6$ sperm/ml)	37.33±5.83	22.01±3.54 †
Sperm Motility (%)	52.56±2.63	81.18±1.19 †
Progressive sperm Motility (%)	31.45±2.41	54.43±2.25 †
Normal Sperm morphology (%)	53.66±2.57	87.33±1.07 †

Values are Mean ± S.E.M

†: Means a highly significant (P<0.001) difference from pre-activation

*No. of infertile patients=20

Table 2: Effect of Ham's F-10 medium supplemented with vitamin E on semen parameters pre and post in vitro sperm activation for infertile patients* with asthenozoospermia

Parameters	Conventional layering technique	
	Pre-activation	Post-activation
Sperm Concentration ($\times 10^6$ sperm/ml)	40.35±6.31	22.75±3.65 †
Sperm Motility (%)	54.00±2.55	86.60±2.07 †
Progressive sperm Motility (%)	31.90±1.66	54.85±1.43 †
Normal Sperm morphology (%)	44.50±3.20	80.25±2.09 †

Values are Mean ± S.E.M
†: Means a highly significance (P<0.001) different from pre-activation
*No. of infertile patients=20

Table 3: Effect of Ham's F-10 medium supplemented with vitamin C on semen parameters pre and post in vitro sperm activation for infertile patients* with asthenozoospermia

Parameters	Conventional layering technique	
	Pre-activation	Post-activation
Sperm Concentration ($\times 10^6$ sperm/ml)	48.12±4.52	25.63±1.41 †
Sperm Motility (%)	54.12±3.63	88.34±1.29 †
Progressive sperm Motility (%)	38.72±1.45	67.90±1.38 †
Normal Sperm morphology (%)	42.41±2.50	83.40±1.81 †

Values are Mean ± S.E.M
†: Means a highly significance (P<0.001) different from pre-activation
*No. of infertile patients=20

Discussion

The results of the present study are in a good agreement with results obtained by Zavos et al. [16] who reported that layering technique significantly has higher percentage of recovery of motile spermatozoa, progressive motile spermatozoa, higher DNA integrity, and numbers of pregnancies than other sperm preparation method. However, it was assessed that sperm concentration, motility, morphology, viability, membrane integrity, acrosomal status, ROS formation, and chromatin

maturity results could be evaluated with usefulness of sperm preparation techniques [17]. In addition, Sills *et al.* [18] mentioned that the selection of sperm preparation methods depend on the quality of the ejaculates. The ejaculates with ROS production by spermatozoa and leukocytes should not be separated by centrifugation method due to severely spermatozoa damage.

It was noticed that the problem caused by ROS can resolve by performed directly from liquefied semen underneath an overlay of culture medium and aspirate directly from the interface region with total number of spermatozoa recovered [19]. However, Aitken and Clarkson [20] suggested that poor IUI outcome may be related to improper preparation techniques with release of harmful ROS as well as the separation of motile and active sperm from the rest of the semen can significantly improve pregnancy rates [21]. Furthermore, it was reported that common laboratory factors like centrifugation, washing, temperature fluctuation, and processing delay harmfully affect semen quality both positively and negatively due to direct influence of laboratory interventions on the cytoskeletal assemblies of sperm [22].

Many studies focus on isolating the population of infertile men who are most likely to benefit from vitamin E supplementation. Potential populations could include men with increased ROS levels, increased DNA fragmentation or asthenozoospermia. The supplementation of sperm preparation medium with vitamins C and E may reduce free radical production and decrease ROS induced DNA damage in patients with poor sperm quality. This in turn may provide a greater chance of successful fertilization, as there is an inverse correlation between percentage of sperm with DNA fragmentation and fertilization rates in vitro with both IVF [23] and ICSI [24]. There is also possibility that oral administration of ascorbate may facilitate a reduction in induced DNA damage, although this is an area that requires further investigation before any firm conclusions can be drawn. As a result, vitamin E enhanced has the potential to help numerous couples that suffer from male infertility.

The dosage and duration of vitamin E supplementation also needs to be explored and optimized. While, vitamin C act as a scavenger of a wide range of ROS [25], which explains its ability to successfully counteract the effects of free radicals both in terms of induced DNA damage and ROS production. It has previously been shown to be the major antioxidant in seminal plasma of fertile men, contributing up to 65% of the total chain breaking antioxidant capacity [26]. The concentration of this antioxidant in seminal plasma is 10 times greater than the concentration found in blood plasma. The study by Moilanen and Hovatta suggested that vitamin E is less possible to have a protective role given that its seminal plasma concentrations were below the beneficial levels. The study found that this vitamin concentration in the spermatozoal membrane rather than in the seminal plasma is positively correlated with improved sperm parameters [27].

The combination of vitamins could substantially reduce ROS levels and impair its normal physiologic function. The current study outlines the beneficial effects of antioxidant supplementation on induced DNA damage. Previous studies have shown that vitamin E affords sperm cells some protection from oxidative attack both in vivo [28] and in vitro [29] studies. The oral administration of vitamin E has also been shown to lead

to a significant improvement in the in vitro function of human sperm as assessed using the zona-binding test and has been suggested as a treatment for ROS-associated male infertility [30].

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