Effect of L- carnitine and Co Enzyme Q10 Addition to SMART Pro- Medium on Human Sperm Parameters During *In Vitro* Sperm Activation

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Abstract

This study was designed to assess the effect of different concentrations of L-carnitine and Co enzyme Q10 (COQ10) supplied to SMART Pro- medium on human sperm motility and sperm grade activity (progressive, non-progressive and immotile) during *in vitro* sperm activation.

Eighty seven samples of semen of infertile and fertile men were randomly collected from the subjects at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al- Nahrain University. The mean age of the men was (32.034 ± 0.57) years with mean duration of infertility (4.644 ± 0.25) years. Seminal fluid analyses were done involving macroscopic and microscopic examinations according to WHO criteria. Swim-up technique was dependent for *in vitro* sperm activation , the washed samples were divided before using centrifugation swim-up technique into 3 groups: control group (G1) without L-carnitine and CoQ10. While, in G2, G3 two concentrations of L-carnitine and CoQ10 (20 µg , and 40 µg) were used. Moreover, all groups within post-activation enriched with SMART-Pro media. The sperm motility and sperm grade activity were evaluated after the addition of L-carnitine and COQ10. The results showed a significant ($P<0.05$) differences were showed in sperm motility and sperm grade activity of post-activation when compared with the pre-activation. In G2 and G3, with dose of (20 µg and 40 µg) of L- carnitine and CoQ10, the results showed a significant ($P<0.05$) increase in the percentages of sperm motility, progressive sperm motility in spite of the increase in the percentages of sperm motility, progressive sperm motility in G3 compared to G2, there was non significant ($P>0.05$) differences between G2 and G3. The results for non-progressive sperm motility% and total progressive sperm motility millions/ mL. for 40 µg (G3) groups were significantly ($P< 0.05$) decreased when compared to control group (G1) with non-significant ($P>0.05$) differences between 20µg (G2) group, with significant ($P< 0.05$) decreased in immotile sperm percentage in post-activation when compared to the pre-activation. From the results of the study, it can be concluded that the addition of 40 µg of L-carnitine and CoQ10 to washed sperms can improve sperm motility and sperm grade activity *in vitro*. Data were analyzed statistically using complete randomized design (CRD) (one way ANOVA).

Key words: L-carnitine, COQ10, SMART medium, sperm function parameters.

Introduction:

Infertility is the inability of a sexually active non contraception couple to achieve pregnancy in one year [1] . Infertility can be due to the woman, the man, or both; primary or secondary. In primary infertility, the couples have never been able to conceive; while in secondary infertility there is difficulty in conceiving after having conceived (either carried the pregnancy to term or had a miscarriage) [2]. A male factor is solely responsible in about 20% of infertile couples and contributory in another 30- 40%, if a male infertility factor is present, it is almost always defined by the finding of an abnormal semen analysis for the assessment of male fertility [3]. Male infertility is due to the complete absence of sperm in the ejaculate and is relatively uncommon. Male sub fertility can be due to low numbers of sperm, a low percentage of sperm with effective progressive movement or abnormalities in the sperm-s ability to fertilize an egg [4]. Conventional semen analysis continues to be the only routine test to diagnose male factor infertility, although semen parameters have a limited power to predict spontaneous or assisted conception [5]. Semen analysis became the most important examination to be performed in the approach to the infertile couples [6]. Basically, sperm count, motility and percentage of MNS are conventional criteria for semen quality [7]. Motility is the prime functional parameter that determines the fertilizing ability of spermatozoa [8]. Sperm motility gives a measure of the integrity of the sperm axoneme and tail structures as well as the metabolic machinery of the mitochondria [9].

L-carnitine play important role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and the spermatogenic process [10]. Coenzyme Q10 is a non enzymatic antioxidant that is related to low-density lipoproteins and protects against peroxidative damage. Since it is an energy-promoting agent, it also enhances sperm motility [11], and a major cellular antioxidant [12]. The aims of the study were to investigate the effect of different concentrations of (L-carnitine and COQ10) supplied to culture medium on sperm motility and sperm grade activity during *in vitro* sperm activation.

Materials and methods:

All samples of semen were collected after (3-5) days of abstinence directly in a clean, dry and sterile disposable Petri- dish by masturbation in a private and quite room adjacent to the semen analysis laboratory. Each Petri-dish was labeled with the person name, age abstinence period and time of sample collection. The specimens were placed in an incubator at 37ºC for 30 minutes to allow liquefaction [13]. The liquefied semen is then carefully mixed for few seconds to homogenize , and then subjected to both macroscopic and microscopic examinations within one hour from collection according to WHO manual (1999) [14]. Each sample was divided into three groups, after being centrifuged once with 2500 rpm for 6 minutes, the supernatant was discarded and then gently (1mL) of SMART Pro-medium was added only for the first group (G1), SMART Pro + 20 µg of L-carnitine and CoQ10 for the second group (G2) and SMART Pro + 40 µg of L-carnitine and CoQ10 for the third group (G3). The samples placed in the incubator an inclined position and sperm parameters were read after 30 minutes. The standard of WHO (2010) [1]. is used to record details of the semen analysis results.

Statistical analysis:

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Sperm parameters pre- and post *in vitro* sperm activation and groups of infertile men were analyzed using complete randomized design (CRD) (one way ANOVA).

Results:

Eighty-seven males were involved in this study. The mean age of subjects was 32.034 ± 0.57 years old with the range 21-51 years. However, the mean duration of infertility was 4.644 ± 0.25 years with the range 1-12 years.
Macroscopic and microscopic parameters of semen analyses (SA) for subjects are shown in (Table1). In this table the sperm motility (%), progressive sperm motility (%) were within normal ranges according to the criteria of WHO (2010) [1]. The results for all semen samples of post activation after using SMART- Pro medium supplemented with 20 µg (G2) or 40 µg (G3) of both L-carnitine and CoQ10) revealed a significant (P< 0.05) increase in the percentage of sperm motility, progressive sperm motility as compared to pre- activation with a significant (P< 0.05) decrease in non-progressive sperm motility %, immotile sperm% and total progressive sperm motility millions/ mL. However, 40 µg (G3) group provides improvement in the percentages of sperm motility, progressive motility, immotile sperm as compared with 20µg (G2) group with non-significant (P>0.05) differences.

Table (1) : Sperm motility for men involved in this study (Mean ±S.E).

Parameters		Mean ±S.E	WHO(2010) criteria
Sperm motility (%)		67.974 ± 1.18	≥ 40%
Sperm grade activity (%)	Progressive motility	43.256 ± 1.57	≥ 32%
	Non-progressive motility	24.527 ± 1.07	
	Immotile sperm	32.182 ± 1.20	
Total Progressive sperm millions/mL		68.044 ± 3.97	

Table (2) : SMART- Pro medium enriched with two concentrations of L-carnitine and CoQ10 for all subjects (mean ± S.E).

Parameters		Pre - activation	Post- activation		
			Control G1	20 µg G2	40 µg G3
Sperm motility (%)		67.698 ^{b} ±1.25	98.821 ^{a} ±0.45	98.263 ^{a} ±1.14	98.406 ^{a} ±0.40
Sperm grade activity (%)	Progressive sperm motility	43.257 ^{b} ±1.68	91.066 ^{a} ±1.31	93.182 ^{a} ±1.00	94.589 ^{a} ±0.87
	Non Progressive sperm motility	24.236 ^{a} ±1.14	7.755 ^{b} ±1.07	5.939 ^{bc} ±0.83	5.188 ^{c} ±0.86
	Immotile sperm	32.470 ^{a} ±1.27	1.179 ^{b} ±0.45	0.638 ^{b} ±0.28	0.223 ^{b} ±0.07
Total Progressive sperm millions/mL		67.807 ^{a} ±4.19	28.614 ^{b} ±2.55	24.205 ^{bc} ±2.42	22.754 ^{c} ±2.44

*Number of subjects = (87).
*Means with different superscripts within each ro are significantly different (P<0.05).
*Means with similar superscripts within each row a non significantly different (P>0.05).

Discussion:

In this study, a combination of the coenzyme Q10 (CoQ10) and L-carnitine (low concentration 20 µg and high concentration 40 µg) were supplemented to SMART-Pro medium to improve sperm parameters *in vitro*. A well improvement in the sperm parameters was showed for treated groups as compared to the pre activation group.

Several factors have been participated for enhancing outcome of *in vitro* activation through sperm activity including presence of mitochondria with adequate numbers and normal activity [15], presence of ATP [16]. Normal and adequate gene expression for protein synthesis [17], integrity of plasma membrane [18], reduction level of ROS [19], presence of CoQ10 [20], L- carnitine contents and environment of SMART- Pro medium [21]. A sperm separation technique should produce a high yield of motile spermatozoa, should be quick, easy to handle, low in cost and it should avoid damaging the cells and eliminate dead spermatozoa, bacteria and reactive oxygen species (ROS) [22] .
Centrifugation swim-up technique was used in this study, the SUP technique relies on the ability of the motile spermatozoa to "swim u into the culture medium, while slow

immotile sperm remain behind, along with other components in the semen pellet [23]. In the present study, sperm centrifugation was selected as a method for *in vitro* sperm activation depending of the results of the Shaaban (2007) [24]. SMART-Pro medium was used in current study for *in vitro* sperm culture. It is reported that the successful IVF has been highly dependent on the pre-fertilization events, which is affected by the composition of culture medium [25]. Trounson and Caro remarked that the successful human and animal IVF is dependent on maintenance of suitable culture conditions for gametes and early developed embryos [26]. As in the study, sperm motility increased with the use of *in-vitro* culture because of their aqueous nature with lower viscosity than of seminal plasma resulted in making spermatozoa move more freely [27].

SMART- Pro medium which invented by Fakhrildin and Flayyih (2011) [28], medium was prepared based on Ringer solution supplemented with two essential additives Sodium lactate and/or pyruvate and human serum albumin (HSA) (5% or 10%). It was known that the albumin play a major role in physiology and metabolism of spermatozoa [29]. It is noticed in the current study that the use of *in vitro* SMART-Pro medium a significant ($P< 0.05$) improvement in sperm parameters , as shown in (control) compared to pre-activation. Enhancement sperm parameters may be considered as normal response for sperm physiology after the removal of seminal plasma, pus cells and agglutinated spermatozoa using sperm preparation techniques. Furthermore, it was reported that only the active motile sperms will swim-up to the upper layer of culture medium *in vitro* human sperm activation [30, 22].

In the present study, percentage of sperm motility and progressive sperm motility were significantly ($P< 0.05$) increased in the groups supplemented with L – carnitine and CoQ10 post-activation as compared to the pre-activation group. This is in agreement with a study by Balercia *et al*. (2005) [31] , and was in agreement with other studies that reported a significant increase in the sperm motility when L- carnitine was added to ejaculated human spermatozoa [32].The results also are in agreement with Xuan *et al*. who documented that maturation, respiration, motility and fertility are dependent on the progressive increase in epididymal and spermatozoa carnitine, critical for mitochondrial fatty acid oxidation, as sperm pass from the caput of the epididymis showed that L- carnitine increased the number of viable sperm cells [33]. This may be due to the effect of L- carnitine on sperm metabolism as well as its antioxidant properties [34,35].

The antioxidant property of LC may have an influence on sperm motility [36]. Also, CoQ10 is an essential part of the cellular energy production machinery; cellular antioxidant. So, severely deficient in the level of CoQ10 showed a reduced ATP in cells [37]. Moreover, the exogenous administration of CoQ10 has been reported to improve sperm motility and this administration has been reported to have a positive role in the treatment of asthenozoospermia [38]. Because of its bio energetic and an antioxidant role CoQ10 has been suggested to be involved in male infertility[39]. Moreover, contents of SMART medium [28] was a very important actor for enhancing sperm motility.

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