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A new stimulatory method for activation of sperm functions using Fertilaid in male infertility

Shukir R. Hamashareef[1], Estabraq A. Alwasiti[1*], Yildiz H. Tahseen[2], Maysoon Mahmood Hussein[3], Hayder A. Al-Aubaidy[4]

Abstract

This study aims to determine the effect of Fertilaid and L- carnitine on the oxidative stress and seminal fluid parameters of the infertile male. One hundred male participants were involved in this study. Seminal fluid samples were collected in duplicates and they were exposed into two forms of in vitro treatments: Fertilaid, and traditional L carnitine. The in vitro sperm stimulation by L Carnitine and Fertilaid in nutrient mixture Ham's F-10 have a significant increase in the activity of the motile sperm (sperm/million) (30.99 for grade A; 30.25 for grade B and 29.68 for grade C) as compared to sperm activation using L carnitine (sperm/million) (27.03 for grade A; 27.40 for grade B and 22.16 for grade C), $p<0.05$. Seminal levels of Malondialdehyde (1.02 nmol/ml) was significantly elevated in the infertile samples as compared to the fertile samples (0.94 nmol/ml), $P<0.05$. This was associated with a significant reduction in the seminal levels of reduced glutathione in the infertile samples (5.67 nmol/ml) as compared to the fertile samples (7.10 nmol/ml), $p<0.01$. In conclusion, in vitro semen activation by fertilaid showed a significant increase in the overall semen parameters as compared to the activation by L. Carnitine.

Keywords: Fertilaid; Hams-F10; L Carnitine; Malondialdehyde; Glutathione; Male infertility

*Corresponding author email: estabraqalwasiti@yahoo.com
[1]College of medicine, Al-Nahrain University, Baghdad, Iraq
[2]College of Medicine, University of Kirkuk, Kirkuk, Iraq
[3]Ministry of Health and environment of Iraq, Baghdad, Iraq
[4]School of Life Sciences, La Trobe University, Bundoora, VIC, Australia
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Introduction

Infertility is the inability of a sexually active non-contraceptive couple to achieve pregnancy in one year [1]. There are two type of infertility (primary and secondary) [2]. There is a relationship between infertility and reactive oxygen species (ROS). Spermatozoa are particularly vulnerable to the harmful effects of ROS [3]. Oxidative stress affects their activity, damages DNA structure, and accelerates apoptosis, all of which consequently decrease their numbers, motility and development of normal morphology, and impairs function. This leads to disturbances in fertility [4]. The main cellular sources of ROS in the semen are immature sperm cells and white blood cells. The protective antioxidant system in the semen is composed of enzymes [5], as well as no enzymatic substances, which closely interact with each other to ensure optimal protection against ROS. Non–enzymatic antioxidants include vitamins A, E, C, and B complex, and L

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Carnitine, and micronutrients such as zinc, selenium [6]. This study uses a new stimulatory method by combining L carnitine and Fertilaid. L Carnitine is a small water-soluble molecule that plays an important role in fat metabolisms. It also plays a fundamental role in the normal mitochondrial oxidation of fatty acids and generation of Acyl-CoA esters [7]. Fertilaid, on the other hand, is formulated by Amos Grunebaum, MD [8]. Fertilaid for men is a non-prescription dietary supplement that combines a potent array of vitamins, antioxidants, and a blend of herbal ingredients, as well as the amino acid, L Carnitine. It is hypothesides that Fertilaid may enhances sperm health in several vital categories, including overall sperm count, sperm motility, and normal sperm morphology.

Method

This study was carried out in the Soran Private Hospital and Pharma Main Lab in Erbil, Iraq. The period of study was from April, 2016 to August, 2017. One-hundred male participants were involved in this study. After liquefaction of 25 fertile sample which considered as control normal human semen underwent macroscopical and microscopical examination. Semen samples were collected by masturbation after 3 days of sexual abstinence and examined after liquefaction for 30 min at 37°C. Semen parameters include (volume, pH, concentration, and motility), were evaluated according to the guideline of the World Health Organization guidelines [9].

Markers of oxidative stress

Malondialdehyde (MDA), a marker of the degree of lipid peroxidation, was estimated in the seminal plasma according to the Shara et.al. method in 1992 [10]. Reduced glutathione (GSH) levels were also measured in seminal plasma using spectrophotometric analysis after deproteinization of the seminal plasma samples, as discussed in Akerboom & Sies, (1981). Absorbance values were compared with standard curves from known amounts of GSH standards [11].

Those 75 infertile seminal samples were divided into two groups (A and B) for in vitro activation. Group A was activated with L Carnitine, while group B was activated with Ham's F10 culture media. Seminal analysis was conducted after the activation to look for the changes in the seminal parameters and the markers of oxidative stress.

Statistical analysis

The collected data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22) for windows. The quantitative statistics of answers were analyzed as frequency, percentage, means and standard deviation were used for numerical variables Student's t-test was used to compare values after activation. Differences between values were considered significant at $P<0.05$ and high significant at $P<0.001$.

Research Article doi: 10.18081/2333-5106/020-02/65-72

Results

Table 1 shows semen parameters of the participants enrolled in the study. The mean value of sperm number in fertile male group was (55.44 sperm/million), and (50.59 sperm/million) in the infertile male group, P<0.001. There was non- significant difference in the volume of the seminal samples in the infertile sample (3.32 ml) as compared to the fertile samples (3.21 ml), P>0.05.

Table 1.

Semen parameters (means + standard deviation) in the two study groups (Infertile, and Fertile). P value was significant at or below 0.05.

Parameters	Groups	No.	Mean	SD	P-value(*)
Sperm count (sperm/Million)	Infertile	75	50.59	1.39	P=0.001
	Fertile	25	55.44	2.08	HS
Volume (sperm/ml)	Infertile	75	3.32	0.15	P=0.054
	Fertile	25	3.21	0.26	NS

Table 2 shows seminal motility response to in vitro treatment with Fertilaid according the motility grade (A, B, C, and D) sperm/million, in the infertile study group.

Table 2.

Mean values of oxidative stress parameters of Malondialdehyde (MDA) and reduced Glutatione (GSH) in all participants of this study before *in vitro* treatment

Parameters	Groups	No.	Mean	Std. Deviation	P-value(*)
GSH (nmol/ml)	Infertile	75	5.67	0.18	P=0.01
	Fertile	25	7.10	0.15	
MDA (nmol/ml)	Infertile	75	1.02	0.07	P=0.05
	Fertile	25	0.94	0.07	

(*) Statistical Hypothesis are based on Levene test and Student t-test.

Table 3 shows seminal motility response to in vitro treatment with L Carnitine according the motility grade (A, B, C, and D) sperm/million, in the infertile study group.

Table 3.

Summary statistics for studied Motility Grades (A, B, C, and D) in Seminal Fluid Post - Activation with L Carnitine

Parameter	Grades	No.	Mean	SD
Activations	A + L Carnitine	31	27.03	2.17
	B + L Carnitine	22	27.40	2.16
	C + L Carnitine	17	22.16	2.91
	D + L Carnitine	5	23.47	2.70

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In addition, there was a significant elevation in the seminal levels of malondialdehyde (1.02 nmol/ml) in the infertile samples as compared to the fertile samples (0.94 nmol/ml), P<0.05. This was associated with a significant reduction in the seminal levels of reduced glutathione in the infertile samples (5.67 nmol/ml) as compared to the fertile samples (7.10 nmol/ml), p<0.01.
Mean values of oxidative stress parameters of Malondialdehyde (MDA) and reduced Glutatione (GSH) in all participants of this study before in vitro treatment

Table 4.

Compares the results of the oxidative stress markers in response to the two in vitro treatment methods.

Parameters	Groups	No.	Mean	Std. Deviation	P-value(*)
GSH (nmol/ml)	Infertile	75	5.67	0.18	P=0.01
	Fertile	25	7.10	0.15	
MDA (nmol/ml)	Infertile	75	1.02	0.07	P=0.05
	Fertile	25	0.94	0.07	

(*) Statistical Hypothesis are based on Levene test and Student t-test.

There was a significant rise in the levels of reduced glutathione (6.3 nmol/ml) in the Fertilaid treated groups as compared to the L Carnitine treated group (5.9 nmol/ml), P<0.05. Malondialdehyde was not significant among the two study groups.

Table 5.

Mean values of Malondialdehyde (MDA) and reduced Glutatione (GSH) in Infertile Seminal Fluid following in vitro post activation with L Carnitine and Fertilaid.

Parameters	Groups	No.	Mean	Std. Deviation	P-value (*)
GSH (nmol/ml)	L carnitine	75	5.9	0.2	P=0.05
	Fertilaid	57	6.30	0.5	
MDA (nmol/ml)	L carnitine	75	0.99	0.05	P=0.9
	Fertilaid	75	0.97	0.02	

(*) Statistical Hypothesis are based on Levene test and Student t-test.

Discussion

From the results of this study, we believe that seminal activation by 0.5 mg Fertilaid with 1.5ml of Ham's F10 media after incubation and centrifugation, increased the sperm motility and gave excellent improvement in progressive sperm motility grade (A) and grade (B), as compared to the L Carnitine treated group. Fertilaid for men is a doctor-developed male fertility-enhancing formula that has been previously shown to improve sperm quality in trying-to-conceive women. In our study the Fertilaid effects on sperm motility was more than L carnitine's effects. Fertilaid may promote the development of healthy sperm as well as increased sperm motility. Fertilaid contains a mixure of L Carnitine, a blend of antioxidants, vitamin E, vitamin C, Selenium, and Zinc [12].

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Vitamin supplementation may be beneficial in improving seminal fluid parameters as showed in previous studies [13]. The addition of vitamin B12 at 2.50 mg/mL to bovine semen in vitro increased sperm motility [14]. Vitamin C basically secreted from seminal vesicles during ejaculation to protect sperm from endogenous oxidative DNA damage [15]. Our results agreed with the results of Lewis et al. who recommended adding low molecular weight antioxidants like ascorbate to sperm during preparation for ART [16].

In this study, infertile male sperm motility was significantly improved by the addition of Fertilaid that contain Vitamin E which is in agreement with other data who found that supplementation of vitamin E with the media significantly enhanced survival rate of spermatozoa [17].

This study believed that the in vitro activation have a positive effect on sperm motility which is shown in table 3, it was concluded that infertile samples, activated by 0.5 mg L Carnitine with 1.5ml volume of Ham's F10 media after incubation and centrifugation increased the sperm motility. These results were in agreement with that of Lee JW who use the stimulation treatment for sperm activation [18].

In our study the effect of Fertilaid on MDA concentration is more than the effect L carnitine on MDA concentration. The abnormal levels of MDA concentration which is shown in table (4) in seminal fluid may increase levels of lipid peroxidation which lead to decrease fertility [19]. This is a characteristic of oxidative stress and it shows an imbalance of the balance between ROS and antioxidants [20]. Lipid peroxidation was responsible for the reduction in membrane fluidity, aggregation and rearrangement of the phospholipids bilayer [21].

In our study. We have noticed that the effects of Fertilaid on levels of GSH is more significant than the effects of L carnitine. The potential role of antioxidants in ameliorating such damage has begun to be examined with studies involving, GSH *in vitro* [22]. The antioxidant GSH levels in the infertile seminal fluid was found to be significantly low as compared to the control fertile subjects, table (4). This is consistent with other studies, which showed abnormal low levels of GSH in the seminal fluid of infertile and sub-fertile groups as compared to a healthy fertile group [23]. However, other resulted data observed, the abnormal levels of GSH in the semen of infertile subjects clearly indicate that the spermatozoa of infertile subjects were exposed to abnormal antioxidant level [24].

Competing interests

The authors declare that they have no competing interests.

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