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A Comparative Study of Fructose, Zinc and Copper Levels in Seminal Plasma in Fertile and Infertile Men

Nawal K. Hussain[1] *PhD*, **Shahlaa S. Rzoqi** [2] *MSc*, **Ali W. Numan**[3] *MSc*, **Dina T. Ali**[1]

[1]Institute of Embryo Research and Infertility Treatment, AL- Nahrain University, [2]Medical Technical Institute/Presidency of the Foundation of Technical Education/Erbil University, [3]Dept. of Chemistry, College of Science, Baghdad University

Abstract

Background Human semen contains high concentrations of fructose, zinc (Zn) and copper (Cu) in bound and ionic forms for Zn and Cu. The presence of abnormal levels of fructose and those trace elements may affect spermatogenesis with regard to production, maturation, motility and fertilizing capacity of the spermatozoa.

Objective To evaluate the levels of fructose, Zn and Cu in seminal plasma in different groups of male infertility and to correlate their concentrations with various sperm parameters.

Methods The concentrations of fructose, Zn and Cu were measured in 114 semen samples from normozoospermic, oligozoospermic, astheno-zoospermic, and azoospermic men using the electrothermal-atomic absorption spectrometry for Zn and Cu determination. The concentration of fructose in seminal plasma was determined with a spectrophotometric method, using the resorcinol method**.**

Results Results of the present study showed that there was an inverse relationship between fructose levels and sperm count. The mean value of seminal plasma fructose concentrations was significantly increased ($p \leq 0.001$) in the three groups of infertile male subjects (azoospermia, asthenozoospermia and oligozoospermia) than in fertile males. The mean value of seminal plasma Zn concentrations was significantly decreased ($p \leq 0.001$) in the three groups of infertile male subjects (azoospermia, asthenozoospermia and oligozoospermia) than in fertile males. A good correlation in a positive direction was noted between the sperm count and seminal plasma Zn concentration. There was significant decrease in seminal plasma Cu concentration between asthenozoospermia and control groups ($p \leq 0.05$) and insignificant increase in oligozoospermic patients.

Conclusions On the basis of the observations of the present study, seminal fructose, zinc and copper may contribute to fertility through their effects on various semen parameters.

Key words Male infertility, fructose, zinc, copper.

Introduction

Infertility has often been defined as failure to achieve pregnancy within one year of unprotected intercourse. Infertility has multiple causes and consequences depending on the gender, sexual history, life style of society and cultural background of people it affects [(1)]. Infertility affects about 8-12% of the world's population and in about half of cases men are either the single cause of or contribute the couple's infertility [(2)].

Fructose concentration, because it is considered a measure of seminal vesicle function, has been studied in great detail. Studies indicate that there is a wide variation in fructose concentration [(3)], and

this concentration can be a function of a number of factors, including time since collection and the age of the donor [4]. Fructose is an important source of energy for the sperm, and, hence, measurements of fructose concentration in whole semen can change over time as a result of fructolysis, the primary source of lactic acid in semen [5,6]. Fructose is also likely involved in protein complexes, particularly in coagulated semen [7].

In fact trace elements calcium, magnesium, copper, selenium, and zinc play very vital role in affecting various parameters of semen. Among trace elements increasing evidence of a direct relationship of zinc was found with seminal parameters [8]. Zinc (Zn) in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa [9]. It may also have an antibacterial function and protect the testis against the degenerative changes [10]. It may play a regulatory role in the process of capacitation and acrosome reaction [11].

The total zinc content in semen from mammals is high, and zinc has been found to be critical to **s**permatogenesis. Deficiency of zinc is associated with hypogonadism and insufficient development of secondary sex characteristics in humans [12], and can cause atrophy of the somniferous tubules in the rat and hence failure in spermatogenesis [13].

Zinc is excreted from the prostate as a low-molecular weight complex with citrate. After ejaculation, 50% is redistributed and bound to medium- and high-molecular weight compounds from the seminal vesicles [14].

Testicular Zn is critical for normal spermatogenesis and for sperm physiology; it preserves genomic integrity in the sperm and stabilizes attachment of sperm head to tail [13].

Copper is an important element for numerous metalloenzymes and metallo-proteins that are involved in energy or antioxidant metabolism. However, in its ionic form and at high level, this trace element rapidly becomes toxic to a variety of cells, including spermatozoa [15]. The present study was designed to evaluate seminal plasma levels of zinc, copper and fructose to correlate their concentrations with various sperm parameters among fertile and infertile male subjects.

Method

This study was carried out at the Institute of Embryo Research and Infertility Treatment, AL-Nahrain University, Baghdad, during the period from Sep. 2008 to Mar. 2009. Eighty six primary infertile male subjects, who had regular unprotected intercourse for at least one year without conception with their partners, aged (25-40) years were included in this study. Patients had no infections, traumatic abnormalities which could be implicated in the development of infertility. At first clinic attendance, a detailed background history and physical examination were done on both husband and wife.

Semen specimens from all infertile patients were collected into sterile polystyrene jars after an abstinence period of 3 to 5 days. Macroscopic and microscopic examination of semen was performed according to WHO recommendations [16]. A portion of each semen sample was examined for sperm count, motility and morphologic features. Infertile male patients were then divided into the following three groups count /motility and/or morphology, WHO criteria, 1992 [17].

Group I: Azoospermic (sperm concentration = zero, n=28),

Group II: Oligozoospermic (sperm concentration < 20 ×10 /ml, n= 30), and

Group III: Asthenozoosperic (sperm motility < 50%, n=28).

Twenty eight fertile males whose partners had conceived within one year and having sperm concentration more than 20 million/ml with motility and morphology more than 50% were selected from general population and taken as normospermic control group.

After liquefaction, the seminal plasma was collected after centrifugation at 3000 rpm for 15-20 minutes. The supernatant seminal plasma was transferred in fresh tubes and stored at -20 °C until assay. Seminal Zn and Cu measurements were performed by the electrothermal-atomic absorption spectrometry (AAS) method. The concentration of fructose in seminal plasma was determined with a spectrophotometric method, using the resorsinol method.

Statistical analysis

Descriptive statistics were represented as mean and SE. Statistical differences were analyzed using Independent sample-test when we had 2 groups, but ANOVA was used when we had more than 2 groups. P-values were considered statistically significant ($p < 0.05$). Pearson correlation was used to assess the relationship between studied variables.

Results

Table 1 summarizes the mean (±SEM) value on seminal plasma levels of Fructose, Cu and Zn in the three groups of infertile male subjects (azoospermia, oligozoospermia and asthenozoospermia) and in fertile control group.

The mean (±SEM) value of seminal plasma fructose concentrations was significantly increased in the three groups of infertile male subjects (azoospermia, asthenozoospermia and oligozoospermia) than in fertile males ($p < 0.001$, Table 1). There were no significant differences in seminal plasma Cu concentration between the azoospermia and the oligozoospermia groups of infertile male subjects and in fertile control group (Table 1), but there was significant decrease in seminal plasma Cu concentration between astheno-zoospermia group and control as shown in Table 2. The mean (±SEM) value of seminal plasma Zn concentrations was significantly decreased in the three groups of infertile male subjects (azoospermia, asthenozoospermia and oligozoospermia) than in fertile male (Table 1).

The results of Table 3 appeared positive and significant ($p < 0.001$) correlation between sperm concentration and total progressive motility. Meanwhile, negative weak and non significant ($p > 0.05$) correlations were assessed between sperm concentration and each of fructose and cupper. However, positive weak and non significant ($p > 0.05$) correlations were noticed between total progressive sperm and each of fructose and cupper. On the other hand, zinc appeared positive weak and non significant ($p > 0.05$) correlations with both sperm concentration and total progressive sperm. In contrast, zinc presented negative weak and non significant ($p > 0.05$) correlations with each of fructose and cupper.

Table 1: Fructose, Cu and Zn Concentration in Seminal Plasma in Three Groups of Infertile Males and Fertile Control Group

		N	Mean± Std. Error	Sig.
Sperm concentration	Control	28	73.393±5.692	≤ 0.001**
	Asthenozoospermia	28	34.964±1.597	
	Oligozoospermia	30	11.833±1.349	
Total progressive	Control	28	87.179±12.135	≤ 0.001**
	Asthenozoospermia	28	10.786±1.147	
	Oligozoospermia	30	5.667±1.015	
Fructose	Control	28	210.643±13.651	≤ 0.001**
	Azoospermia	28	392.500±17.170	
	Asthenozoospermia	28	266.536±17.096	
	Oligozoospermia	30	261.133±21.260	
Cu	Control	28	0.047±0.006	> 0.05^{NS}
	Azoospermia	28	0.044±0.010	
	Asthenozoospermia	28	0.025±0.004	
	Oligozoospermia	30	0.051±0.008	
Zn	Control	28	157.593±11.785	≤ 0.05*
	Azoospermia	28	105.893±6.664	
	Asthenozoospermia	28	132.250±11.590	
	Oligozoospermia	30	125.367±10.370	

The values are expressed as Mean (±SEM).
NS = no statistical significance $p > 0.05$.
* = statistical significance $p < 0.05$.
** = highly statistical significance $p < 0.001$.

Table 2: Cu and Zn Concentration in Seminal Plasma in Asthenozoospermia Group of Infertile Males and Fertile Control Group

	Study groups	Mean± Std. Error	Sig.
Sperm concentration	Control	73.393±5.692	≤0.001**
	Asthenozoospermia	34.964±1.597	
Total progressive	Control	87.179±12.135	≤0.001**
	Asthenozoospermia	10.786±1.147	
Fructose	Control	210.643±13.651	≤0.05*
	Asthenozoospermia	266.536±17.096	
Cu	Control	0.047±0.006	≤0.05*
	Asthenozoospermia	0.025±0.004	
Zn	Control	157.593±11.785	>0.05^{NS}
	Asthenozoospermia	132.250±11.590	

The values are expressed as Mean (±SEM).
NS = no statistical significance $p > 0.05$.
* = statistical significance $p \leq 0.05$*.
** = highly statistical significance $p \leq 0.001$**.

Table 3: Pearson Correlation coefficient among studied variables

		Sperm concentration	Total progressive	Fructose	Cu	Zn
Sperm concentration	r	1	0.508	-0.047	-0.237	0.108
	p	0.000	≤ 0.001**	> 0.05^{NS}	> 0.05^{NS}	> 0.05^{NS}
Total progressive	r		1	0.072	0.008	0.073
	p		0.000	> 0.05^{NS}	> 0.05^{NS}	> 0.05^{NS}
Fructose	r			1	0.056	-0.131
	p			0.000	> 0.05^{NS}	> 0.05^{NS}
Cu	r				1	-0.181
	p				0.000	> 0.05^{NS}
Zn	r					1
	p					0.000

NS= no statistical significance p >0.05.
* = statistical significance $p \leq 0.05$*.
** = highly statistical significance $p \leq 0.001$**.

Discussion

The normal function of seminal vesicle is essential for sustaining fertility. Decreased function of seminal vesicle affects the semen coagulation, sperm motility, stability of sperm chromatin, and semen immunoprotection. One of the most important markers for the seminal vesicular function is the concentration of fructose in seminal plasma [18].

The results of the present study showed that there was an inverse relationship between fructose levels and sperm concentration. Similar results have been reported by Manivannan et al [19]. However this finding was conflicting with others [20,21]. The lowest values of seminal fructose presented may be due to the increase of the process of fructolysis. Furthermore, the decrease of fructose concentration was significantly positively correlated with motile sperm concentration [18].

It appears that the abnormal concentrations of this substance are related to disturbances in the secretory activity of the seminal vesicles [22].

In this study, there was a significant low level of seminal plasma zinc levels in oligozoospermic and azoospermic males. Similar results have been reported by Hasan et al [23]. Our results are also incompatible with several studies [24]. A good correlation in a positive direction was noted between the sperm count and seminal plasma zinc concentration. This element has been shown to be highly important for conception, successful implantation and pregnancy outcome [25, 26]. Zinc is present at high concentrations in the seminal fluid and there is evidence that it may act *in vivo* as a scavenger of excessive O^{-2} production by defective spermatozoa and/or leukocytes in semen after ejaculation [27]. There is evidence that zinc plays a vital role in the physiology of spermatozoa and spermatogenesis. Specifically, Bedwal et al reported that shrinkage of seminiferous tubules Zinc is an essential nutritional component. A potential benefit of zinc supplementation for immuno-logical competence is currently widely discussed.

Zinc is present at high concentrations in the seminal fluid, and may play a multifaceted role in sperm functional properties. It has been suggested as being an important anti-inflammatory factor, and also to be involved in sperm oxidative metabolism [29].

A clinical study demonstrated that adult males experimentally deprived of zinc showed a disturbance of testosterone

synthesis in the Leydig cell. Since zinc plays an important role in 5α reductase enzyme that is necessary for the conversion of testosterone into biologically active form 5α dihydro-testosterone [23]. The authors concluded that adequate seminal concentration of the Zn is required for normal sperm function. It has been demonstrated that Zn in human semen is derived from the prostate [12]. Zn appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation [30].

The results of this study also showed that Cu concentration was significantly decreased in seminal plasma of asthenozoospermic patients while insignificantly increased in oligospermic. This result is compatible with that observed by others [15]. Copper is an essential trace element that plays an important role in several enzymes such as cytochrome oxidase, ferroxidase, superoxide dismutase and spermin oxidase. Human spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also possess a significant ability to generate a reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide. Superoxide dismutase (Cu-metalloenzyme) protects human spermatozoa from this peroxidative damage [15]. Liang Lu et al 2009 [31] suggested that Cu^{2+} can affect male reproductive function through T-type Ca^{2+} channels. *Invitro* studies, Roblero et al [32] have demonstrated the effect of Cu^{2+} on the motility viability, acrosom reaction and fertilizing capacity of human spermatozoa.

On the basis of the present observations and those of others seminal fructose, zinc and copper may contribute to fertility through their effects on various semen parameters. Adequate seminal plasma concentration of fructose, Zn and Cu are required for normal sperm function and that high toxic concentrations of Zn and Cu in seminal plasma are apparently related to defective motility of sperm in infertile males. It seems that the estimation of seminal fructose, Zn and Cu may help in the investigation and treatment of infertile males.

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Correspondence to: Dr. Nawal Kh. Hussain
E-mail: nawalkhiry@yahoo.com
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