MALE INFERTILITY AND PHYSIOLOGICAL ROLE OF ZINC

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Abstract

Background: Male infertility is the inability to conceive a baby in the absence of female causes. Most causes reflect an abnormal sperm count or quality. Zinc is a component of semen and plays an important role in the process of reproduction and sexual maturation.

Objectives: To asses the relationship between concentration of zinc in serum, seminal plasma and semen quality among infertile and fertile men to clarify the possible impact of zinc in male reproductive system.

Methods: Fifty-eight male (infertile group) partners who were undergoing investigation for infertility and thirty-seven men (fertile group) whose wife were pregnant and have normal sexual life studied as a control group.
Seminal fluid analysis, reproductive hormones (luteinizing hormone, follicular stimulating hormone and testosterone) were analyzed by using radioimmunoassay method (RIA) while serum and seminal plasma zinc concentration were determined by using colorimetric methods.

Results: All semen properties (count, motility and morphology) for the infertile group were lower than for the fertile group. No significant differences were found in the levels of L.H. and F.S.H. between the two groups while testosterone levels were significantly lower in the infertile men group than in the fertile group ($p<0.005$).
It was also found that the mean level of serum and seminal plasma zinc concentration were significantly lower in the infertile group compared to the fertile group ($p<0.0001$). No correlation was found between the concentrations of zinc in serum and seminal plasma in both groups. A lack of correlation was also found between zinc concentrations in serum and seminal plasma with semen properties.

Conclusion: The study revealed the importance of zinc in fertility through its direct and its indirect effects on spermatogenesis.

Keywords: Male infertility, Zinc, semen, spermatogenesis

IRAQI J MED SCI, 2005; VOL. 4 (1): 67-71

Introduction

Many recent studies have indicated an increasing prevalence of various abnormalities of the reproductive system in human males[1]. Male infertility is as the inability to conceive a baby after 1 year of unprotected sex in the absence of female causes.
Most causes of male infertility reflect an abnormal sperm count or quality. Although it only takes one sperm to fertilize an egg, in an average ejaculate a man will eject nearly 200 million sperm. However, because of the natural barriers in the female reproductive tract only about 40 sperm will ever reach the vicinity of an egg.
There is a strong correlation between the number of sperm in an ejaculate and fertility. In about 90% of the cases of a low sperm count, the reason is deficient sperm production. Unfortunately, in about 90% of cases, the cause for the decreased sperm formation cannot be identified. Other causes of male infertility may include ductal obstruction, ejaculatory dysfunctions, and infections or disorders of the accessory glands[2].
Prostate gland is the largest accessory gland of the male reproductive tract and there is a good deal of research being conducted on the prostate. Yet it

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Received 14th April 2004: Accepted 29th November 2004

remains one of the least understood structures in the body. One area of study concentrates on the major function of the gland, the production and the secretion of minerals that play an important role in prostate and the reproductive system functions.

Zinc is a component of semen and it is though that at least 1 mg of zinc is excreted by an ejaculum. Zinc plays an important role in the processes of fertility, reproduction and sexual maturation[3]. At a biochemical level, zinc's primary role as a component of zinc metalloenzymes, many enzymes of the cell nucleus involved with genetic information transfer and cellular replication are metallonezymes of zinc. Zinc also participates in RNA metabolism, prostatic fluid and ocular tissues contain the highest levels of zinc in the body[4].

The present study aimed at clarification of the interrelationship of serum and semen zinc levels, in both men with a history of infertility and men with no history of infertility. Also, investigate the possible role of seminal zinc in the regulation of human spermatozoa functions.

Subjects & Methods

Subject selection:

Iraqi men in the age range of 20 to 50 years with no apparent chronic or acute disease were selected for the study, 37 men of known fertility and 58 infertile men were studied.

The infertile men were unable to be fathers of children for at least 1 year; their wives were considered fertile based on extensive gynecologic evaluations. At the first interview for the infertile men, a complete history was obtained, especially with respect to any history of sexual dysfunction. Physical examination was performed to exclude patients with chromosomal abnormalities. None of the subjects were on drug treatment or on especial diet and none of the subjects used vitamin supplements.

Procedures:

1. Semen collection: semen analysis were performed on three separate specimens for each subject, samples were collected after 2-3 days of sexual abstinence by masturbation into polyethylene containers.
2. Serum collection: Blood was drawn into sterile, disposable plastic syringes. After allowing 30 minutes, for blood clotting, the serum was separated from blood cells by centrifugation at 900 g for 5 minutes at room temperature. The serum was decanted and stored in a metal-free polypropylene tube at $–20C^o$ until required.
3. Seminal plasma collection: The semen was put into plain tube and after one hour the seminal plasma was separated by centrifugation at 3000 rpm at room temperature for 10 minutes. The seminal plasma was decanted and stored in a metal- free polypropylene tube at -20 ^oC until required.
4. Hormonal Assay: Basic endocrine measurements includes serum LH and FSH will testosterone by radioimmunoassay method (RIA)[5] from (CIS Bio International, groups ORIS, B.P. 32-91192. GIF-SUR-Yvette Cedex-France).
5. Zinc analysis: Zinc analysis was done by Jenway 6100 spectrophotometer (model 6100 serial No. 3000; frequency 50/60 Hz, UK. England) according to the methods described by Shibata, S., who said pyridylazo compounds of phenol derivatives are sensitive chromogenic reagents for the analysis of zinc and many other metals[6].

An aliquot of 50μL of serum was mixed with 1ml of the reagent and 10μL of seminal plasma was mixed with 1ml of the same reagent. All tubes were mixed and incubated for 10min at 25^{o}C. Then measured against blank at 560nm using (Cat. No. 0033, Giesse Diagnostics, C.F. 03084750581, P. IVAO

11572610077, Reg. Soc. N. 1103178. Trib- Roma).

Statistical Analysis

The results are presented as (mean ± standard deviation) for better comparison with relevant data in literature

The results within the groups were also presented, as ranges, and the significance of the differences between the groups were calculated using student's t-test two-tailed.

The Pearson's correlations (r, p) and regression equation were calculated between each of the measured parameters to assess the shape of relationship based on the highest (r) value obtained.

Results

The mean±standard deviation and range of semen quality (counts, motility, and morphology), serum reproductive hormones (LH, FSH, Testosterone) and serum zinc and semen zinc are listed in table I.

Table 1: Parameters of semen quality, reproductive hormones, and Zinc concentrations in fertile and infertile men groups

Item	Fertile men group (n=37)		Infertile men group (n=58)		P-value
	Mean±SD	Range	Mean±SD	Range	
Count (60-150x10^6/ml)	96.6±28.3	57-154	31.28±30.0	0-98	S
Motility (>60%)	75.1±11.7	55-95	15.0±12.3	0-60	S
Morphology (>70%)	84.3±5.5	70-90	56.9±31.8	0-90	S
LH (1-5mIu/ml)	3.5±1.0	1.5-5.0	4.7±3.9	0.9-23	Ns
FSH (1-9mIu/ml)	6.0±1.7	2.8-9.8	6.4±6.0	0.9-34.5	NS
Testosterone (8.2-34.6nmol/L)	22.5±4.5	15.8-33.0	18.0±8.2	7.0-44.0	S
Serum Zn (70-120µg/dl)	118.7±16.8	90.6-162	97.5±26.9	36-147.4	S
Seminal plasma Zn (1.2-3.9nmol/L**)**	3.1±0.4	2.5-3.8	2.1±0.6	0.39-2.7	S

The parameters of semen quality were significantly lower in the infertile men group than in the fertile men group (P<0.0001). No significant differences were found in the mean concentrations of LH and FSH in serum, when fertile men group was compared with infertile men group, but the mean serum testosterone concentration was significantly lower in the infertile men group than in the fertile men group (P<0.005).

The results revealed that serum zinc levels in infertile men group were lower than in the fertile men group (P<0.0001). In addition, seminal plasma zinc levels were lower in the infertile men group than in the fertile men group (P<0.0001).

From table 2, there was no significant correlation between serum zinc and the parameters of semen quality in both groups. Moreover, no significant correlation between semen zinc and the parameters of semen quality in both groups.

Table 2: Values of simple correlation coefficient (r) between Zinc concentration and parameters of semen quality for both groups

Item	Fertile Men Group (n=37)		Infertile Men Group (n=58)	
	Serum Zn	Seminal Plasma Zn	Serum Zn	Seminal Plasma Zn
Count	-0.098	0.058	-0.006	-0.041
Motility	-0.285	0.12	-0.025	-0.187
Morphology	-0.071	-0.368	-0.179	0.099

No correlation was found between semen zinc and serum zinc level in the fertile men group (r=0.075) nor in the infertile men group (r=0.120).

Discussion

Our data are in agreement with those of Bonde et al[7], who reported that not sperm concentration but sperm quality determines fertilizing capacity of spermatozoa.

Although, the most cases of male infertility are nonendocrine in origin. However, routine evaluation of hormonal parameter is not warranted unless sperm density is extremely low or there is clinical suspicion of an endocrinopathy. A scrotal varicocele is the most common causative finding in infertile men[8]. To explain the abnormalities in spermatogenesis with varicocele, the most point have been proposed was the abnormal blood flow can interfere with testosterone production, which in turn can interfere with sperm production and this in agreement with our results that about 25% of infertile men had varicocele.

We did not find any difference in the our data and in those previously published in the literature for that the serum zinc and semen zinc levels were significantly lower in infertile patients than fertile males[9], in contrast to other reports, that unable to find a significant difference in serum and semen zinc levels between fertile and infertile men[10,11].

The lack of correlation between zinc concentration and semen quality found in our study suggests that biochemical complexity of seminal fluid attempts to perform such simple correlations between seminal plasma component and andrological parameters are likely to produce inconsistent results. These effects include no significant correlation between the total amount of zinc per ejaculate and sperm quality[12] with no statistically significant correlation between zinc concentration and the motile sperm concentration[13].

However, our results are in contrast to other studies that showed zinc-related decrease in human semen quality[14,15]. Most previous results of colorimetric methods and the present method found that the results obtained using the proposed method were not statistically different from those obtained by atomic absorption spectrophotometry[16,17].

The high level of zinc found in semen is due primarily to the secretions of the prostate gland and reflects prostatic stores. Serum zinc may be a reasonable indicator of zinc status. The lack of correlation between serum zinc and semen zinc found in our study suggests that mild zinc deficiency may lower serum zinc while the larger prostate zinc stores remain unaffected. However, we obtained 25% had varicocele, which are enlargement of the internal spermatic veins that drain the testes.

The significant decrease of the zinc in seminal plasma of varicocele men were significantly lower than in the normal subjects .This decrease indicated an impairment of the prostatic function or secretion due to decreases the availability of oxygen and nutrient required for sperm live.

Citrate is part of the circular chain of cellular respiration, known as the Krebs's cycle. The Krebs's cycle is the process in which a sequence of enzymatic reactions involving the metabolism of carbon chains of sugars, fatty acids, and amino acids to yield carbon dioxide, water, and high-energy phosphate bonds. The Krebs's cycle provides a major source of adenosine triphosphate energy and produce molecules that are starting point for a number of vital metabolic pathways for the cell, citrate that been chelated with zinc and their evidence that zinc required for oxygen consumption by sperm.

Varicocele decreases the concentration of zinc and decreases the availability of oxygen for live sperm. Further studies to elucidate the significance of zinc and other factors presents in seminal plasma in different types of causes of male infertility for the functional properties of human spermatozoa appear to be of importance since such studies may give hints to new ways of regulating male fertility.

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