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Research Article

## A Comparison of Some Biochemical Parameters in Sera and Semen of Fertile Male in Fallujah City

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**Abstract:** The aim of the objective study was to inspect the concentrations of some biochemical parameters. Total protein, the enzymes activities and some metals in sera and semen of normal, healthy men were determined and the relationships between these parameters in each fluid were examined. The samples (n=32) were collected between April to September 2013 from Fallujah city. The results showed that there is a significant positive correlation was found between serum and semen in all parameters except total protein and RNase activity.

**Keywords:** Biochemical parameters, sera & semen, fertile male, Fallujah city.

### INTRODUCTION

The seminal fluid which is released during male ejaculation, usually consists of approximately (2-5) ml with an average range of spermatozoa of round (20) million per milliliter of ejaculation<sup>1</sup>. This fluid appears as viscous, creamy, slightly, yellowish or grayish fluid, which at first goes through the clotting process and then becomes more liquid<sup>2</sup>. The fluid composed of the sperm and seminal plasma comprises 95% of semen volume it a safe surrounding for spermatozoa, at pH (7.3-7.8) it has protected spermatozoa from the acidic environment of the vagina<sup>3, 4</sup>. It is a complex mixture rich in biochemical components like serum of organic and inorganic molecules that contribute to sperm function and thus to the events leading to fertilization<sup>5</sup>. Seminal plasma has a high concentration of fructose sugar that provides nutrition and energy for mitochondria in spermatozoa.<sup>6</sup>

The prostate gland is the main source of the acid phosphatase, zinc, and magnesium found in the ejaculate. Seminal vesicle secretions are rich in fructose<sup>1</sup>. These components are affected by the properties of the transfer vehicle and its communications with the surrounding fluids. Several studies have compared healthy subjects (normal sperm count) to male infertility (oligospermia or azoospermia). Zinc is an element essential for normal function of the male reproductive system where zinc is dependent in the numerous of biochemical mechanisms, including more than 200 enzymes in the body.

The deficiency of zinc is associated with decreased testosterone level and sperm count, an adequate amount of  $Zn^{+2}$  ensures proper sperm production and their motility. Copper is an essential element for all known living organisms including humans and other animals at low levels of intake. Copper products are used as components of larger systems, such as building, motor vehicle and the telecom wire. The role of copper in the male reproductive capacity appears to be the largely unknown issue, the copper is a naturally occurring trace element that is essential for some metabolic processes. The main part of the protein found in semen derive from the seminal vesicles, although albumin is generally of prostatic origin. Even though the exact role of protein in seminal plasma is not known, but there are some evidence that proteins and amino acids play a role in sperm survival.

Adenosine deaminase (ADA) or adenosine aminohydrolase (EC 3.5.4.4) is the important enzyme in a purine metabolism. It is involved in a breakdown of dietary adenosine as well as those produced from the turnover of nucleic acids in tissues. This ubiquitous enzyme has been found in the wide variety of microorganisms, bacteria, plants and vertebrates with a highly conserved of amino acid sequence in addition, it is present in all mammalian cells.

RNase (polyribonucleotide 2-oligonucleotide-hydrolase, (EC: 3.1.4.22) are widely distributed in nature. They all possess one common property, namely the cleavage of the P-O5' bond of RNA on the 3' side of cytidine and uridine residues via intermolecular transphosphorylation<sup>7</sup>. RNases of mammals and other vertebrates constitute a large superfamily of enzymes having greatly divers' functions other than a simple digestive role.

These enzymes are widely distributed in various organs and body fluids. Phosphatases have been classified as acid phosphatase (EC 3.1.3.2) and alkaline phosphatase EC (3.1.3.1) according to optimum pH required for their catalytic activity<sup>8,9</sup>. Dephosphorylation and phosphorylation of seminal plasma proteins and sperm are regulated by the opposing activities of phosphatases and kinases<sup>10,11</sup>.

## AIM

The present study examined total protein, the enzymes (ADA, Alk. RNase, Acp. & ALP) activity and some metals ( $Zn^{+2}$ ,  $Cu^{+2}$ ) in normal, healthy men and the relationships between these parameters in blood serum and seminal plasma were examined

## MATERIALS AND METHODS

Semen sample and sera were obtained from thirty two fertile men with an age range (23-48 years) ( $36.2 \pm 5.4$ , mean  $\pm$  SD) who were residing in Fallujah city, Iraq. A complete medical history with physical examination was made by doctors of the advisory clinic in Fallujah General Hospital.

**Samples collection of the study:** Excluded smoker persons and injured the diabetes disease or any chronic disease as well as the person who is drinking wine.

**Collection of Seminal Fluid:** Seminal fluid was produced by masturbation after three to five days of the sexual abstinence. Samples were left for 20 to 60 minutes for liquefaction to occur. Then semen quality was evaluated by using two parameters: macroscopically and microscopic examination of semen analysis using world health organization criteria<sup>1</sup>.

**Collection of blood samples:** Blood samples were collected from participants; 10 ml of venous blood was collected into plastic tubes and stored in sterile polyethylene tubes. After extracting serum samples of whole blood, about 5 ml of serum samples was stored at  $-20\text{ }^{\circ}\text{C}$  until the analysis. The sera were used for determination of metals ( $\text{Cu}^{+2}$  and  $\text{Zn}^{+2}$ ) and the other analysis.

**Determination of trace elements concentration:** The trace elements were determined by digesting 1 ml of sample with 5ml of an acid mixture ( $\text{HNO}_3$ :  $\text{HClO}_4$ ) in a volume ratio of 6:1 in a glass tube. Then, the concentration of trace elements ( $\text{Cu}^{+2}$  and  $\text{Zn}^{+2}$ ) was measured by using Atomic absorption Spectrophotometer GBC 933 plus (Shimadzu / Japan), with air-acetylene flame and hollow cathode lamp<sup>12</sup>.

**Determination of total protein:** A sensitivity of the biuret method by Janairo<sup>13</sup> was used to determine total proteins in serum of blood and seminal plasma using Bovine Serum Albumin (BSA) as a standard absorption of the colored solution was measured at  $\lambda = 545\text{ nm}$ .

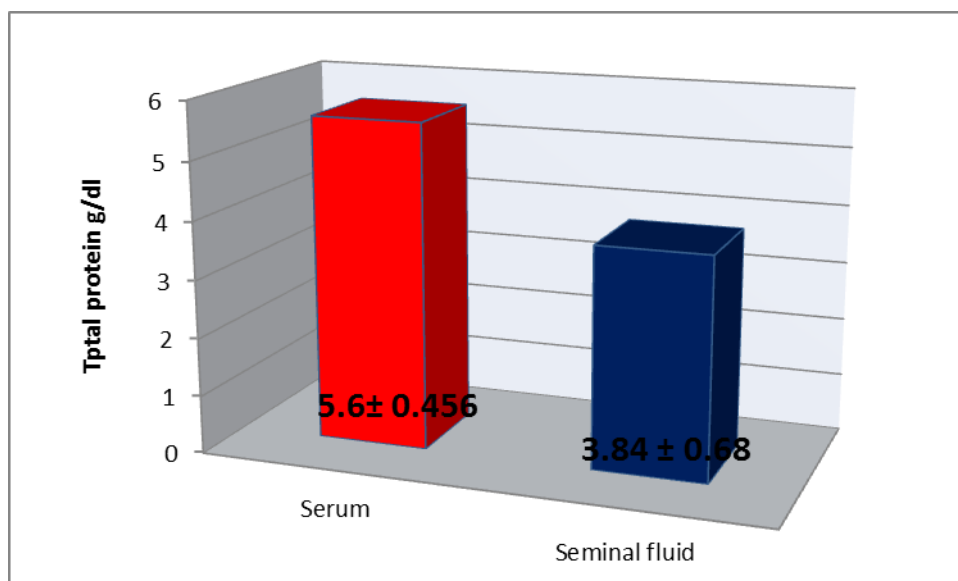
**Determination of the different enzymatic activity:** Alkaline RNase activity in sera & seminal plasma expressed of uniting where one unite of RNase is defined as, "*one Kunitz unit of RNase A is the amount of enzyme required to cause an increase in absorbance of 1.0 at 260 nm at 37°C (pH 8.0) when yeast RNA is hydrolyzed to oligonucleotides. 50 units = 1 Kunitz unit*"<sup>14</sup>. The method with some modification—Adenosine deaminase (ADA) activity was determined by Giusti and Galanti method<sup>15</sup>.

Adenosine deaminase activity was expressed using U/l where: "*One U is can be defined as the amount of enzyme that produces a certain amount of the enzymatic activity, that is, an amount that catalyzes a conversion of 1 micro mole of substrate per minute*"—Alkaline phosphatase (Alp) activity was determined by the calorimetric method, it was expressed by kind and king unit (KAU/l) which is defined as "*is the amount of enzyme which, in the given*"<sup>16</sup> condition liberates 1 mg of phenol in 15 minutes at 37 °C. Acp activity is extremely labile at room temperature. The stabilization of the enzyme can only be achieved by acidifying with acetate buffer also activity was determined according to a modified method of Hillmann<sup>17</sup> Acid phosphatase activity was expressed by U/l the unit is defined as "*the amount of an enzyme that produces a certain amount of the enzymatic activity, that is, an amount that catalyzes a conversion of 1 micro mole of substrate per minute*".

## RESULTS

The concentration of total protein in serum and semen was shown in **Figure (1)**.

Total protein in blood serum was within the normal range, the correlation between total protein concentrations in both fluids was non-significant ( $p \geq 0.05$ ). Significant differences in the concentrations of elements (Zn and Cu) in seminal fluid and serum of fertile male were shown in Table (1). Trace elements that determined in serum and semen (**Table 1**),  $\text{Cu}^{+2}$  is significant in the comparison, the concentration of  $\text{Zn}^{+2}$  in serum ( $1.081 \pm 0.162$ ), seminal fluid ( $16.022 \pm 3.516$ ) (Mean  $\pm$  SD).



**Figure 1:** Total protein (g/dl) in serum and seminal fluid, mean  $\pm$  SD

**Table 1:** Concentrations of trace elements in sera & seminal fluid

Biochemical Parameter	Serum (Mean $\pm$ SD)	Seminal fluid (Mean $\pm$ SD)
Zn <sup>+2</sup> ( $\mu$ g/ml)	1.081 $\pm$ 0.162	16.022 $\pm$ 3.516*
Cu <sup>+2</sup> ( $\mu$ g/ml)	0.025 $\pm$ 0.012*	0.005 $\pm$ 0.002

Where \* is a highly significant increase ( $P \leq 0.01$ )

All enzymes in this study (**Table 2**) were significant when compares its activity in serum with it in seminal fluid except RNase non-significant.

**Table 2:** Activity of enzyme in sera & seminal fluid

Biochemical Parameter	Serum (Mean $\pm$ SD)	Seminal fluid (Mean $\pm$ SD)
Alk. RNase(U/l)	7.005 $\pm$ 2.408**	10.830 $\pm$ 3.035**
ADA(U/l)	18.856 $\pm$ 3.441*	5.276 $\pm$ 1.367
Acid phosphatase (U/l)	88.306 $\pm$ 15.902	23703.030 $\pm$ 6592.016*
Alk. Phosphatase (kAU/dl)	7.697 $\pm$ 1.539	25.133 $\pm$ 3.409*

Where \* is a highly significant ( $P \leq 0.01$ ), \*\* is a nonsignificant ( $P \geq 0.05$ )

## DISCUSSION

Compared to the peripheral blood, semen contains high concentrations of chemicals and substances originating from the male reproductive organs, their component can be regarded as potential biomarkers for disease diagnosis and evaluate the function<sup>28</sup>. Seminal plasma proteins could serve as important biomarkers for male infertility<sup>4</sup>. The concentrations of seminal protein have been used as markers of seminal vesicle function<sup>18</sup>. The human seminal fluid is a viscous mixture of spermatozoa and fluid from seminiferous tubules, accessory glands and the epididymis (seminal vesicles, the prostate, and bulbourethral glands).<sup>19</sup>.

Trace elements may affect the male reproductive system indirectly, when they act on the neuroendocrine system or directly when they target specific reproductive organs. These effects can be long lasting and irreversible if Sertoli cells are disrupted through fetal development. Copper can act as both a pro-oxidant and an antioxidant. Free radicals occur naturally in the body this will lead to damage cell membrane, contribute to the development of a number of health problems and diseases,  $\text{Cu}^{+2}$  neutralize free radicals, or scavenges and may reduce or help prevent some of the damage they cause<sup>20</sup>. Also the trace element like copper has been suggested as a highly toxic element for sperm and can affect sperm motility in humans. The significant difference ( $p \leq 0.01$ ) (increase) in the activity of this enzyme was found in compared between the serum with semen. RNase the superfamily members are secretory proteins typically composed of a signal peptide and a mature peptide<sup>21</sup>, RNase in semen, sequestered within microvesicles derived from apoptotic spermatogenic cells may be the primary macromolecule associated form present, additionally, there may be some other important RNA-associated macromolecules in semen, such as free messenger ribonucleoprotein particles, a subset of which function as global stabilizers /translational suppressors of mRNAs in male germ cells<sup>22</sup>.

The ADA activity in biological fluids must be aware of the concept and the significance of the "2'deoxyadenosine/ adenosine deaminase ratio"<sup>23</sup>. In addition, ADA is present in all mammalian cells and the enzyme primary function in the humans is development, differentiation, and the maturation of the lymphoid system<sup>24</sup>. ADA in serum was statically highly significant compared with its activity in semen ( $P < 0.001$ ). The results of the current study disagree with Feng et al who explained that ADA in seminal plasma has not been found in, MEDLINE database, and their possible physiological roles in male reproduction remain unclear<sup>25</sup>. ADA is an important enzyme in the metabolism of adenosine and immunity system.

Acp are a family of enzymes that belong to the hydrolase class. They are specifically grouped together because of the shared ability to catalyze a hydrolysis of the orthophosphate monoesters under the acidic conditions<sup>26</sup>. The activity of Acp in semen was higher than its serum ( $23703.030 \pm 6592.016$ , mean  $\pm$  SD). This result agreed with<sup>19</sup> who shows that Acp and zinc in semen have been used as prostate markers. The physiological function of Acp may be associated with the liquefaction process of semen<sup>27</sup>. Human Acp has had a considerable impact on tools of clinical investigation and intervention. One particular example is tartrate resistant acid phosphatase, which is detected in the serum in raising amounts accompanying pathological bone resorption<sup>26</sup>. Tsekora *et al.*<sup>28</sup> was found the activity of this enzyme increased by  $\text{Cu}^{+2}$  in *Aspergillu niger*.

Alkaline phosphatase is secreted into the seminal fluid by the prostate and testis in man<sup>29</sup>. The mechanism of the action on spermatozoa is general can be assumed to be inhibition of the sperm phosphodiesterase activity, that resulting in elevation of complementary adenosine monophosphate levels in spermatozoa<sup>30</sup>. In this study, high activity of alkaline phosphatase was noted in seminal plasma with large rang (31.205 to 17.010 KAU/l). Despite extensive research on alkaline phosphatases in the male reproductive tract, their role in reproductive physiology is not clear. Difficulties in understanding the role of alkaline phosphatase originate from the universal presence of alkaline phosphatase in reproductive tissue and its wide substrate specificity<sup>31,32</sup>, but suggest a possible role for alkaline phosphatase in the dephosphorylation of adenosine monophosphate. Tang<sup>31</sup> suggested that alkaline phosphatase may inhibit the glycosylation of sperm surface glycoproteins through the hydrolysis of nucleotide sugars.

## CONCLUSION

Seminal plasma biochemical parameters upon in fertilization occur locally, so that study of such parameter in blood plasma cannot be used as an indicator of what is happening in seminal plasma.

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