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Biochemical Study of Blood Serum and Seminal Plasma of Patients with Oligospermia

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Abstract:

The study began in September 2021 and was extended in September 2021 and extended until May 2022. (172) samples of blood serum and (70) samples of seminal plasma were collected after instructing the patient. Abstain from sexual intercourse for 3 consecutive days. Samples were obtained from Al-Salam Teaching Hospital and specialized laboratories in Mosul city. Samples were divided into two groups: A control group and Oligospermia patients were diagnosed by specialized doctors. The samples were classified between the groups based on the general semen analysis by CASA device.

The study included measuring the biochemical parameters of serum and seminal plasma for both control and patients groups, which included: estimating the concentrations of vitamins C, E, and D, measuring glutathione (GSH) and malondialdehyde (MDA), measuring some salts and minerals such as calcium (Ca), sodium (Na), potassium(K), and chloride (Cl), as well as measuring the concentrations of some trace elements such as copper (Cu), zinc (Zn), and cobalt (Co), and estimating some proteins such as prostate-specific antigen (PSA), albumin and total protein concentrations.

The study showed that there were significant decrease in the concentration of vitamins C, E, D, and in Zn and GSH concentrations; we noted a significant increase in the concentration of MDA, and in PSA concentration in blood serum of patients group compared to the control group. It was also shown on seminal plasma that there was a significant decrease in the concentration of Na, K, Ca and albumin, while there was a significant increase in Cu, Co and in the concentration of total protein of oligospermia patients compared to control group.

Keywords: Glutathione, Malondialdehyde, Oligospermia, Prostate-specific antigen, Vitamine D.

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Introduction

Infertility is defined according to the reports of the International Committee for Monitoring of Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO), as the failure of the wife to Pregnancy 12 months after unprotected sexual intercourse [1]. It is generally believed that reproductive issues concern women, but infertility affects both men and women [2] where male infertility is defined as a decrease in

the number of spermatozoa (oligospermia), and a decrease in their motility (Low sperm motility), decreased vitality (sperm necrosis), and abnormal sperm formation (teratozoospermia). The majority of cases of infertility occur due to an internal disorder in the testicle. Male infertility can be complete or partial, called infertility [3].
The term oligospermia refers to a low sperm count in the semen with a low concentration of sperm and is a common finding in male infertility. Oftentimes, semen with a low concentration of sperm may show significant abnormalities in the shape of the sperm. In addition, its movement, or it may be the result of endocrine dysfunction, anatomical abnormalities, taking certain medications, or due to environmental exposure [4]. The diagnosis of oligospermia is based on a low sperm count in a semen analysis. Previously, low sperm concentrations were less than 20 million sperm/ml, in which case they are oligosperm, but recently, the World Health Organization re-evaluated sperm criteria. It established a reference point, less than 15 million sperm/ml, consistent with the reference percentage for fertilization in men [2]. Semen is defined as a fluid agglomeration that is divided into cellular and cellular components. Sperm and other cells are suspended, such as classic round cells, and seminal plasma (SP) is the component a cellular semen, which is a heterogeneous complex fluid consisting of the secretions of the testicle, epididymis, and dependent sex glands a pivotal role in fertility and offspring [5]. It has been found that ROS and oxidative stress are closely related to various diseases such as neurodegenerative diseases, aging and male infertility. Hence, antioxidants such as vitamin C, vitamin E, N-acetyl cysteine, L-carnitine and folic acid are regularly used in various treatment regimens to protect cells from damage caused by free radicals. However, due to their over-the-counter availability in abnormally high concentrations as well as the fact that they are commonly added to various food products, patients may be at risk of consuming excessive doses of these compounds, which can be toxic when the antioxidants are defective. Oxidants Excess oxidants stimulate oxidative stress. Different types of active oxygen molecules are generated by inhibiting antioxidant systems [6].

Material and methods

Sample collection and preparation:

The (122) blood samples were collected from patients suffering from oligospermia from Al-Salam Teaching Hospital, Urology Unit, Madinah Al-Munawarah Laboratory and Al-Watan Laboratory for pathological analyzes, knowing that the patients were diagnosed by specialized doctors, and (45) samples were collected from seminal plasma patients from the hospitals and laboratories mentioned above.

Preparation of serum samples:

Blood serum samples were collected according to the instructions of the World Health Organization, as venous blood was drawn from patients and healthy people after sterilization of the area using alcohol sterilizers or Hepatin, then blood samples were placed in plastic tubes and left until the blood clotted for 15 minutes when 37 °C, then placed in a centrifuge for 4 minutes at a speed of 4000 rpm, and the serum was collected in plastic tubes, and the samples were frozen at -20 °C until It is used to measure biochemical variables.

Preparation of seminal plasma samples:

The sample was collected in the laboratory and this is usually done by masturbation after abstaining from sexual intercourse for three consecutive days. After liquefaction of the sample for 15 minutes, centrifugation is carried out for 10 minutes, then the plasma layer is transferred to sterile plastic tubes and frozen at a temperature of -20 degrees Celsius until the specified measurements are taken. Samples of oligospermia patients were selected after performing a sperm count examination in laboratories through microscopic observation and manual counting of sperm, or using a Computer-Assisted Semen Analyser (CASA). The device is kept at a temperature of 37°C [7].

Estimation of biochemical parameters measured in serum and seminal plasma:

Vitamin D determination in serum:

Vitamin D was measured using an Ichroma II device from Boditech, a Korean company that works with (FIA) fluorescence Immunoassay - quantitative determination II.

Estimation of vitamin E in the serum:

The concentration of vitamin E was measured by following the oxidation-reduction reaction called (Emmeric-Engle Reaction), which includes the reaction of oxidation of tocopherol to tocopherol quinone by ferric chloride, which reduces the ferric ion to the ferrous ion, which will form a red complex, to be read The absorbance at wavelength 460 nm, then ferric chloride $FeCl2$ was added and the absorbance was measured at 520 nm to estimate vitamin E [8].

Estimation of vitamin C in the serum:

Vitamin C was measured by the oxidation the ascorbic acid by copper to form dehydroascorbic acid (DHAA) and 2,4-dinitrophenylhydrazine in presence of thiourea, a derivative of bis 2,4- dinitrophenyl hydrazine and with the addition of sulfuric acid, the formed product gives an absorption band at 520 nm [9].

Estimation of the glutathione in the serum:

Serum glutathione was measured using the modified method used by researchers (Sedlak and Lindsay) in 1968, which depends on the use of (Ellmans Reagent) containing DTNB or what is known as (5.5) -Dithio bis 2-nitro benzoic acid), as the reagent reacts with glutathione and is reduced by the SH thiol group of glutathione to form a colored compound, the intensity of its absorption is measured at a wavelength of 412 nm, and the concentration of the formed product depends On the concentration of glutathione in serum [10].

Estimation of malondialdehyde in serum:

The level of lipid peroxide in the blood was estimated by measuring the amount of malondialdehyde as a final product of oxidized fats. The method depends on the interaction between lipid peroxides mainly (Malondialdehyde) and thiobarbituric acid. The intensity of the absorbance is measured at the wavelength 532 nm [11].

Measurement of calcium concentration in seminal plasma:

The concentration of calcium in seminal plasma was measured by using a standard kit from the French company Biolabo according to the method [10].

Measurement of the concentration of sodium, potassium and chloride in seminal plasma:

The concentration of sodium, potassium and chloride in seminal plasma was measured by using a chemistry device (Abbott auto analyzer instrument) from ARCHITECT, the American company.

Measurement of copper in seminal plasma:

Copper in seminal plasma was measured by using an atomic absorption spectrophotometer - flame (novaAA350) from Analytikjene Company.

Measurement of zinc concentration in serum and seminal plasma:

The zinc concentration in the serum of both groups of patients and control subjects was measured using a standard kit from the Italian company LTA of origin. As for the measurement of zinc level in seminal plasma, it is done using an atomic absorption spectrophotometer - flame (Anova A350) from Analytikjene Company.

Measurement of cobalt in seminal plasma:

The level of cobalt in semen plasma was measured using an atomic absorption spectrophotometer - flame (Anova A350) from Analytikjene Company.

Measurement of prostate-specific antigen in serum:

The antigen was measured in the serum using an Ichroma II device from Boditech, a Korean company that works with Fluorescence Immunoassay (FIA) technology - quantitative.

Measurement of albumin concentration in seminal plasma:

The albumin was estimated using the Bromocresol Green method, according to the method used by (Rodkey) in 1965, as it used a standard kit from the French company Biolabo. [10].

Measurement of total protein concentration in seminal plasma:

The total protein concentration in seminal plasma was measured using the Biuret method, and using a Standard Kit from the French company Biolabo [10].

Statistical analysis:

The statistical program SPSS (version 21) was used to statistically analyze the study data and the complete randomized design (CRD) was followed by the one-way analysis of variance. The averages were used for the study data that included more than two variables, and the standard error SE was used for the study criteria mentioned previously, while Duncan's multiple range test was used [12] to compare the means at the level of significance $P \leq 0.05$, and the t-test via the independent t test was used to compare the study criteria included in the statistical analysis which includes two variables.

Results and discussion

Table (1) indicates that there was a significant decrease in the concentration of vitamin D at P≤ 0.01 in serum of oligospermia patients compared to control group, and this agrees with [13] which they noted that the infertility in men may be caused by low levels of vitamin D, and there is a correlation between the amount of motile sperm and progressive motile sperm and the level of vitamin D in the serum, as it has been proven that vitamin D improves sperm motility and induces an acrosome reaction in laboratory tests [14]. The low level of vitamin D concentration may be due to several reasons, including insufficient availability in the diet or lack of exposure to the sun, in addition to lack of absorption, digestive disorders, inflammatory bowel disease, chronic pancreatic insufficiency, cystic fibrosis and people with liver disease Chronic, such as cirrhosis of the liver [15].

It was also found that there was a significant decrease in the concentration of vitamin E at P≤ 0.01 in the serum of oligospermia patients compared to control group and this is consistent with [16], and the reason for the Low concentration of the vitamin is cholestatic liver disease who increased the risk of developing vitamin E deficiency, and malnutrition, in most cases, occurs due to a lack of vitamin E due to a condition in which nutrients are not properly digested or absorbed. They include Crohn's disease, liver disease, cystic fibrosis, and some rare genetic disorders. Vitamin E deficiency may also occur due to a low-fat diet [17] as shown in Table (1).

It was found that there was a significant decrease in the concentration of vitamin C at P≤ 0.001 in the serum of oligospermia patients compared to control group, and the researchers stated that the reason for the decrease is due to the state of oxidative stress leading to a decrease in dietary antioxidants prevents free radicals that cause oxidative stress [18], where vitamin C deficiency is more common when there is a decrease in intake or an increase in also in requirements, patients at risk of not getting enough of the vitamin are the elderly, those who suffer from anorexia or who are dieting and patients with inflammatory bowel disease, GERD, or Whipple disease, also, when a person has a wound, vitamin C levels in the blood and tissues decrease, as well as in people with gum disease [19].

Table 1: levels of some biochemical changes in serum of oligospermia patients compared to control group.

Parameters	Mean ± SE		value-P
	Control group	Oligospermia group	
Vitamin D ng/ml	**33.0 ± 3.49**	**21.54 ± 1.25**	**0.01**
Vitamin E ng/ml	**0.89 ± 0.11**	**0.54 ± 0.05**	**0.01**
Vitamin C mg/dl	**0.70 ± 0.03**	**0.56 ± 0.02**	**0.001**
GSH IU/ml	**5.45 ± 0.59**	**3.02 ± 0.18**	**0.001**
MDA mmol/L	**1.41 ± 0.99**	**3.31 ± 0.15**	**0.0001**
Zinc mg/dl	**88.78 ± 3.82**	**68.95 ± 2.73**	**0.0001**

Table (1) indicates a significant decrease in glutathione concentration at P ≤ 0.001 in serum of oligospermia patients compared to the control group. This is consistent with [20] which they showed that primary reducing factors in the body are GSH, and glutathione peroxidase, which also acts as scavenger antioxidants in the testes and epididymis by modulating the sperm membrane, and protecting the fatty components, which protects the ability and motility of sperm [21]. The decrease in the level of glutathione may be due to the lack of raw materials needed for the synthesis of glutathione under oxidative stress such as NADPH resulting from the pentose pathway, which would stimulate glutathione reductase, the enzyme responsible for converting GSH to the active form from the inactive form, The other cause results from liver disease, chronic biliary liver injury, liver cancer, and cardiovascular disease [20].

Table (1) indicates that there was a significant increase in the concentration of malondialdehyde at P ≤ 0.0001 in the serum of oligospermia patients compared to control group, and this is consistent with [22]. MDA, is one of the main products of endoperoxidase, which is degraded in the case of various diseases. As a product of oxidative damage, and a product of oxidative stress that causes an imbalance between the production of free radicals and antioxidant activity, it is possible that the increase in MDA is due to a decrease in antioxidant activity, an increase in the concentration of lipid peroxidation, and a decrease in antioxidant changes and alter the activities of cellular enzymes. And to overcome these oxidative stresses, the lack of antioxidant vitamins, such as vitamin E and ascorbic acid. Oxidative stress damages sperm function, causing structural damage to DNA and acceleration of apoptosis, which consequently leads to low sperm count and decreased fertility that leads to inability to achieve pregnancy or fetal development [23].

The results in Table (1) indicates that there was a significant decrease in the concentration of zinc at P ≤ 0.0001 in serum of oligospermia patients compared with control group, and this is similar to [24]. There are many reasons why zinc is important to men's health and typical examples of this are to support immune function, nurture healthy cell growth, and play a role in maintaining a healthy prostate, sexual health, and testosterone levels. Zinc has been shown to play an important role in reproductive functions. Zinc deficiency is associated with low testicular size, hypogonadism, , insufficient development of secondary sex characteristics in humans, shrinkage of seminiferous tubules, failure of spermatogenesis, growth of the male reproductive glands, and hypogonadism. Infertile men with increased levels of ROS are susceptible to zinc deficiency, and studies indicate that zinc has antioxidant activity so oxidative damage from ROS can lead to increased zinc deficiency [24].

Table (2) indicates that there was a significant decrease in the concentration of zinc in seminal plasma at $P \leq 0.001$ in oligospermia patients compared with control group, and this agrees with [25], where zinc is a necessary trace element for the activities of many enzymes in metabolic pathways, because it plays a critical role in sperm formation and motility and in the fertilization process. The data showed that any change in the concentration of zinc and trace elements Others on a physiological scale are likely to have a negative effect on semen quality, zinc has a protective role in reducing the toxicity of copper and iron. Therefore, the decrease in zinc concentration is a negative indicator of fertility and semen quality. It was also found that there is a negative correlation between the value of reactive oxidants and the level of zinc [26].

Table (2) indicates that there was a significant increase in the levels of cobalt in seminal plasma at $P \leq 0.001$ in oligospermia patients compared with control group, and this agrees with [27] that we show the increase in the concentration of cobalt in seminal plasma and serum is a risk indicator of increased oxidative stress because cobalt and copper are associated with the increase in ROS. As well as the increase of cobalt with a toxic effect on tissues, it was found that the elements play major roles in male fertility, and directly affect the quality of sperm [28].

Table 2: levels of trace elements in seminal plasma of oligospermia patients compared to control group.

Parameters	Mean ± SE		value-P
	Control group	Oligospermia group	
Zinc mg/dl	48.94±3.12	36.22 ± 2.24	0.001
Cobalt mg/dl	0.24± 0.18	0.37 ± 0.14	0.001
Copper mg/dl	0.81± 0.07	2.56 ± 0.16	0.0001

The results of the current study showed that there was a significant increase in the concentration of copper in the seminal plasma at $P \leq 0.0001$ in oligospermia patients compared with control group, and this is in agreement with [28]. The higher levels of copper recorded for infertile men may be due to the exposure of these individuals to sources of copper from the environment from drinking water contaminated with toxic levels of copper [27] as shown in Table (2).

Table (3) indicates that there was a significant decrease in the level of sodium concentration in seminal plasma at $P \leq 0.001$ in oligospermia patients compared with control group, and this consistent with [29]. Sodium and potassium are also found in low concentrations in seminal plasma, which have a significant role in sperm interactions. The researcher points out that the lower volume of semen, the low concentrations of sodium, calcium and potassium, and vice versa. Ion channels in the plasma membrane are essential for regulating sperm activity. In fact, proteins are able to communicate information between the sperm and its surroundings by flowing ions through the protein channel on the cell wall. The sperm membrane potential and intracellular pH are such that ion channels maintain their lowest level of activity under typical physiological conditions and are likely to be activated only in the presence of physiological stimuli. These different channels are used to maintain a constant ion concentration in the environment outside the cells and inside the cells so any difference in sodium concentration is a measure of sperm count [30].

The results of the current study also showed a significant decrease in potassium concentration in seminal plasma at $P \leq 0.01$ of oligospermia patients compared with control group as shown in Table (3), and this agrees with [31], which they explained that mineral components such as sodium, potassium, calcium, magnesium, phosphorous, chlorine and iron play an important role in the performance and maintenance of male fertility, but it has no diagnostic value for disorders of the male reproductive system in the case of azoospermia, and suggested that it does not affect the presence of sperm In the semen or its absence in osmosis, osmosis in the extracellular environment plays a key role in regulating sperm metabolism [30].

Table 3: levels of electrolytes in seminal plasma of oligospermia patients compared to control group.

Electrolytes	Mean ± SE		value-P
	Control group	Oligospermia group	
Sodium mmol/L	119.25 ±2.83	104.51 ±3.22	0.001
Potassium mmol/L	15.02 ±1.54	11.42 ±1.24	0.01
Calcium mmol/L	21.60 ±0.93	17.99 ±1.48	0.01

Table (3) indicates that there was a significant decrease in the calcium concentration in the seminal plasma of oligospermia patients compared with control group at $P \leq 0.01$, and this is consistent with [31]. This study showed that the level of calcium in seminal plasma was significantly lower in men with oligospermia compared to fertile men. The oxidative stress that stimulates DNA damage accelerates the programmed death of reproductive cells, which will lead to a decrease in the number of spermatozoa and thus a decrease in calcium. Calcium is secreted from the prostate and its secretion is regulated by the hormone progesterone. Calcium flows from the cellular vacuoles of the prostate cells to semen, which is transported by a compound called myo-inosetol 1,4,5-triphosphate Tp3[32].

Table (4) shows that there is a significant increase in level of PSA concentration at $P \leq 0.0001$ in seminal plasma of oligospermia patients compared to control group, and this is consistent with [33], the results of this study showed that infertile men had higher PSA values than fertile individuals. Whereas, serum PSA correlates with semen quality in infertile men, and a higher proportion of infertile 40-year-olds had a PSA value greater than 1 ng/ml than men who are fertile at the same age, The available literature still does not provide sufficient evidence regarding the clinical relationship of PSA testing to the diagnostic functioning of infertile men [34].

Table 4: levels of prostate-specific antigen, albumin and total protein in the seminal plasma of oligospermia patients compared to control group.

Parameters	Mean ± SE		value-P
	Control group	Oligospermia group	
PSA ng/ml	0.29 ± 0.01	2.11 ± 0.02	0.0001
Albumin g/dl	22.19 ± 1.79	13.26 ±0.79	0.001
Total protein g/dl	28.2 ± 2.4	37.6 ± 2.4	0.01

Table (4) indicates that there was a significant decrease in albumin concentration in seminal plasma at $P \leq 0.001$ in oligospermia patients compared with control group, and this agrees with [35]. The decrease in albumin is due to its use as an antioxidant that contributes to the capture of many mineral elements that enter the process of forming oxidation factors inside the body, such as free iron and free copper, being one of the preventative antioxidants that are built inside the body, and albumin captures oxidants such as hydroxyl radical And its interaction with unsaturated fats in the sperm walls prevents the apparent decrease in many different antioxidants such as vitamin C, vitamin E. The increase in glutathione and albumin and their decrease with the increase of compounds resulting from oxidation such as MDA and peroxynitrite is a clear indication that oxidative stress is severe in patients with oligospermia. In addition, the results indicated a decrease in nutrients, albumin and protein, and the reason is due to the nutritional and economic status without The average of most of the samples collected, which can increase the severity of the decrease in nutrients, protein and albumin, which appeared clearly to the hospital auditors from whom the samples were taken.

Also, the results in Table (4) indicates that there was a significant increase in total protein concentration in seminal plasma at $P \leq 0.01$ in oligospermia patients compared with control group, and this agrees with [35]. Proteins are secreted from different parts of the male reproductive system, such as the testis, seminal vesicles, prostate, and accessory glands. Certain types of proteins protect sperm from oxidative factors (free radicals). Where it blocks its way and prevents them from destroying the sperm and the cells that produce them, and this leads to the destruction of proteins in the semen, which leads to a decrease in their concentration. Free, as the total protein is not affected by a small decrease in the concentration of a specific protein compared with the high concentration of the total protein [36].

Conclusion

We can concluded the role of some biochemical components in the serum and seminal plasma of oligospermia patients and find out the extent of their effect on fertility and know their relationship to the pathological condition by comparing with the fertile healthy group, as well as we can estimated some antioxidants and vitamins among those with oligospermia and estimated the concentrations of some minerals, salts and some trace elements in serum and seminal plasma for the patients and control groups, and studying the concentrations of proteins, including prostate-specific antigen, albumin and total protein.

References

[1] Deyhoul, N.; Mohamaddoost, T. and Hosseini, M. Infertility related risk factors : a systematic review .int J Womens health reported sci, 5(1), pp 24-29.2017.

[2] Wasilewskia, T. ; Łukaszewicz-Za, M. ; Wasilewskac, J. and Mroczko,M. Biochemistry of infertility. Clinica Chimica Acta, 508, pp185-190. 2020.

[3]Sharma, A. Male infertility; evidences, risk factors, causes. Diagnosis and management in human annals of clinical laboratory research , 5(3), pp188-195.2017.

[4]Prasad, K. and Shailesh, D. Management of oligozoospermia by apatyakar ghrut:a case report. Ilkogretim Online - Elementary Education Online, Vol. 19 (Issue 4): pp. 2762-2765. 2020.

[5]Martinez, H. ; Martinez, E. A. ; Calvete, J. J. ; Peña Vega, F.J. and Roca, j. Seminal plasma: relevant for fertility? International Journal of Molecular Sciences, 22(9), pp4368-**4375**. 2021.

[6]Ralf, H.; Inderpreet, S. S. and Ashok, A. The excessive use of antioxidant therapy: A possible cause of male infertility?. Andrologla. First International Journal of Andrology. 2018. https://doi.org/10.1111/and.13162.

[7]Hashim, H. E .and Hasan, M. K." Mitochondrial Number in Oligo and Azoospermia Male Patients"38(Incms):47–52 ,2021.

[8]Varly, H. ; Gowenlock, A.H.and Bell, M. Practical clinical biochemistry. 5th ed , vol .2 , Harold varly , Great Britin,1976.

[9]Kusay, A. M. Al-Chalabi, and Saba Z. M. Al-Abachi. "Enzymatic Changes in Serum of Benign and Malignant Brain Tumors Patients." Journal of Education and Science 21(1), pp19–30. 2008. doi: 10.33899/edusj.2008.51293.

[10]Burits, C.A. and Ashwood E.R. Teites Fundamental Fundamental of Clinical Chemistry : 4nd ed . W.B Sanders company ,USA.,pp: 302, 751-75. 2006.

[11]Mulish, R.K. ;Al-Nimer, M.S and Al-Zamely, O.M.Y. The level of malondealdehyde after activation with (H2O and CuSO4) and inhibition by disferoximine and molsidomine in the serum patients with acute myocardial infraction Nat. J.of chem, 5. pp139-148. 2022.

[12]Duncan, D.B. Multiple range and multiple F tests biometrics, 11(1),pp1-42. 1955.

[13]Zhu, C. L.; Xu, Q. F.; Li, S. X.; Wei, Y. C.; Zhu, G. C.; Yang, C. and Shi, Y. C. Investigation of serum vitamin D levels in Chinese infertile men. Andrologia, 48(10), pp1261–1266. 2016.

[14]Turan, Ö. D. Vitamin D Level and Infertility. Meandros Medical and Dental Journal, 19(2), pp106–110. 2018.

[15]Mohammed, L. Y.; Jamal, S. A.; Hussein, N. R. and Naqid, I. A. Prevalence of Vitamin D deficiency and associated risk factors among General Populations in Duhok province, Kurdistan Region, Iraq. Journal of Contemporary Medical Sciences, 7(6), pp330–333. 2021.

[16]Majzoub, M. S. S. ; Ab-Rahim, S. and Rajikin, M. H. Vitamin E as an antioxidant in female reproductive health. Antioxidants, 7(2), pp 2-10. 2018.

[17]Teriaky, A.; Mosli, M.; Chandok, N.; Al-Judaibi, B.; Marotta, P. and Qumosani, K. Prevalence of fat-soluble vitamin (A, D, and E) and zinc deficiency in patients with cirrhosis being assessed for liver transplantation. Acta Gastro-Enterologica Belgica, 80(2),pp 237-241. 2017.

[18]Young, I. S. and Woodside, J. V. Antioxidants in health and disease. J Clin Pathol, 54, pp176–186. 2001

[19]Taha, I. G. The Effect of Citric Acid on some Biochemical Parameters in Male Rabbit. Rafidain J Sci. ;27(1), pp10-16. 2018.

[20]Umoinyang, E. P.; Elekima, I. and Onwuli, D. Some Heavy Metals Correlated Negatively with Total Antioxidant Capacity, Glutathione Peroxidase, Fructose, and Testosterone in Seminal Plasma of Oligospermic and Azoospermic Males. Journal of Advances in Medical and Pharmaceutical Sciences, October, 15–27. 2021.

[21]C. M. E. and D. S. Nutrient supplementation: Improving male fertility fourfold. Seminars in Reproductive Medicine, 31(4), pp293–300. 2013.

[22]Al-Hayali, H. L. Effect of Cement Pollution on some Biochemical Parameters in the Blood Serum of Hamam AL-Alil Cement Factory Workers. Rafidain Journal of Science, 20(3), pp 19–28. 2009.

[23]Walczak-Jedrzejowska, R.; Wolski, J. K. and Slowikowska-Hilczer, J. The role of oxidative stress and antioxidants in male fertility. Central European Journal of Urology, 66(1), pp 60–67. 2013.

[24]Fallah, A.; Mohammad-Hasani, A. and Colagar, A. H. Zinc is an essential element for male fertility: A review of zn roles in men's health, germination, sperm quality, and fertilization. Journal of Reproduction and Infertility, 19(2), pp69–77. 2018.

[25]Kasperczyk, A.; Dobrakowski, M.; Horak, S.; Zalejska-Fiolka, J. and Birkner, E. The influence of macro and trace elements on sperm quality. Journal of Trace Elements in Medicine and Biology, 30, pp153–159. 2015.

[26]Chyra-Jach, D.; Kaletka, Z.; Dobrakowski, M.; Machoń-Grecka, A.; Kasperczyk, S.; Bellanti, F.; Birkner, E. and Kasperczyk, A. Levels of Macro- and Trace Elements and Select Cytokines in the Semen of Infertile Men. Biological Trace Element Research, 197(2), pp 431–439. 2020.

[27]Onwuli, D. O. and Ajuru, G. Cobalt and Copper Levels in the Seminal Plasma of Infertile Men Living in Port Harcourt Metropolis. 2(2), pp27–31. 2014.

[28]Guzikowski, W.; Szynkowska, M. I.; Motak-Pochrzęst, H.; Pawlaczyk, A. and Sypniewski, S. Trace elements in seminal plasma of men from infertile couples. Archives of Medical Science, 11(3), pp591–598. 2015.

[29]Hamad, A.W. Sodium, Potassium, Calcium and Copper Levels in Seminal Plasma are Associated with Sperm Quality in Fertile and Infertile Men. Biochem Pharmacol Open Access.;03(04). 2014. doi:10.4172/2167-0501.1000141

[30]Cejudo-Roman, A. ; Pinto, F. M. ; Subirán, N. ; Ravina, C. G.; Fernández-Sánchez, M.; Pérez-Hernández, N.; Pérez, R.; Pacheco, A.; Irazusta, J. and Candenas, L. The Voltage-Gated Sodium Channel Nav1.8 Is Expressed in Human Sperm. PLoS ONE, 8(9), pp1–13.2013.

[31]Sakande, J.; Kabre, E.; Ekue-Ligan, A.; Ouedraogo, H. and Sawadogo, M. Relation entre les anomalies du spermogramme et les constituants biochimiques du liquide séminal de sujets consultant pour hypofertilité masculine à Ouagadougou. International Journal of Biological and Chemical Sciences, 6(3), pp1167–1178. 2012.

[32]Harchegani, A. B.; Rahmani, H.; Tahmasbpour, E. and Shahriary, A. Hyperviscous Semen Causes Poor Sperm Quality and Male Infertility through Induction of Oxidative Stress. Current Urology, 13(1), pp 1–6.2019.

[33]Boeri, L.; Capogrosso, P.; Cazzaniga, W. ;Ventimiglia, E.; Pozzi, E.; Belladelli, F.; Schifano, N.; Candela, L.; Alfano, M.; Pederzoli, F.; Abbate, C.; Montanari, E.; Valsecchi, L.; Papaleo, E.; Viganò, P.; Rovere-Querini, P.; Montorsi, F. and Salonia, A. Infertile Men Have Higher Prostate-specific Antigen Values than Fertile Individuals of Comparable Age[Formula presented]. European Urology, 79(2), pp234–240.2021.

[34]Mao, Y.; Xu, X.; Zheng, X. and Xie, L. Reduced risk of prostate cancer in childless men as compared to fathers: A systematic review and meta-analysis. Scientific Reports, 6(January), pp1–8. 2016.

[35]Almadaly, E. A. ; Farrag, F. A. ; Saadeldin, I. M. ; El-Magd, M. A. and El-Razek, I. M. A. Relationship between total protein concentration of seminal plasma and sperm characteristics of highly fertile, fertile and subfertile Barki ram semen collected by electroejaculation. Small Ruminant Research, 144, pp 90–99. 2016.

[36]Macanovic, B. ; Vucetic, M. and Jankovic, A. Correlation between sperm parameters and protein expression of antioxidative defense enzymes in seminal plasma: A pilot study. Dis Markers. 2015. doi:10.1155/2015/436236.