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# Biochemical Enzymatic Study of Infertility

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**Abstract**: This study dealt with the issue of unexplained infertility in men, as it included the measurement of many biochemical variables in the semen plasma, where all of the variables total cholesterol, albumin, thiol group, manganese and zinc were measured TAC. where the results showed the presence of many significant differences between the measured variables when comparing the disease group With the control group, which indicates the different effects of these variables on patients with unexplained infertility. Also, during this study, the effect of hyaluronidase enzyme was followed up and studied by studying the activity of the enzyme as well as the purification stages of the enzyme and estimating the approximate molecular weight using the technique of gel filtration, ion exchange and electro-migration technique (SDS) This enzyme plays a pivotal role in the fertilization process, and any difference in the effectiveness of this enzyme leads to cases of unexplained infertility**.**

**Keywords:** Unexplained infertility, Hyaluronidase , Cholesterol, Thiol group

## Introduction

Infertility is define as the inability of couples to conceive after a year of marriage without any hindrance and it's percentage affects 10-15 % of married couples. Infertility is diagnosed based on the seminal fluid analysis and parameters that are measured by the seminal fluid (cocentration, appearance, motility of the sperm). The causes of infertility are different including several factors such as hormonal imbalance physiological problems, genetic problems (including the single gene), abnormal chromosome (Babakhanzadeh et al., 2020). one of the enzyme that control this process hyaluronidase (E.C.3.2.1.25 ) are endo - B-N- acetyl hexosaminidase that break down B- 1,4 glycosidic linkages to form tetrasaccharides well based hyaluronidase enzyme is break down hyaluronic acid in to monosaccharides by cleaving it is glycosidic bonds and it is present both in organs ( testis, spleen, skin,eyes, liver, kidneys and placenta) and in body fluids ( tears, blood, and semen) on the surface of sperm and plays arole in the maturation of the sperm (Park et al., 2019).

The semen contains many sperms suspended in the middle of the so-called seminal plasma, and it is released from the accessory glands before and after the ejaculation process. (Juyena & Stelletta, 2012) Therefore, semen is considered not only a carrier for the sperm, but also provides them with protection and nourishment during the period of movement in the female reproductive system (Asadpour, 2012).Semen plasma consists of many different biochemical components such as glucose, protein, lipids, cholesterol and a large number of intracellular enzymes, antioxidants and mineral elements (Tvrdá *et al* ,2021) which are very important for sperm function and metabolism (El-Beshbishy et al., 2013).

The process of estimating the biochemical components and enzymes, including the enzyme hyalurinase, is one of the important recommendations to determine the quality and efficiency of semen, because it indicates the function of sperm and damage a number of them and cause the occurrence of so-called unexplained infertility



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(Bhandari et al, 1998), where enzymes play a large and very important role in the process of sperm penetration of the egg and the occurrence of the fertilization process As well as the movement of the sperm, and it gives the sperm the energy needed for life, movement and the fertile period (Köster et al 1986).

## Method

In this research , 34 sample of seminal fluid were collected by the intubation process and with abstinence for aperiod of 3 days from normal people and patients suffering from unexplained infertility who has diagnosed by aspecialist doctor and their ages ranged from 25-30 years. The samples were divided in two parts. The first section is to determenation biochemical parameters and the second part is the isolation of the hyaluronidase enzyme.

### Patient and Controle

The study group consisted of 200 males devided to two group 50 normal and 150 patients. Patient who had attended the endrology clinic for diagnosis of unexplained infertility (normal volum, motility, sperm cell account, spermcell density account, and shap). Collection the sample from reviewers f national laboraties. According insruction word heath organazation (WHO).

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### Sample Collection and Preservation

Seminal fluid collected on the same day.seminal fluid (2-6) ml were collectin by masturabatin on laboratory, after at least 3 days of sexual abstience after (15min) liquefecation were centrifuged at 600xg for 10 min to separate the spermatozoa from the seminal plasma. The each part storage in fresh tube at -20 c.

### Determination the Activity of the Seminal Fluid Hyaluronidase Enzyme

The effectiveness of hyaluronidase was measured according to the method (Okunade & Murthy, 2002) with modification where sodium tetraborate was used instead of potassium tetraborate.

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### Measurement the Activity Hyalorondase Enzyme in Crude and Seminal Plasma Basic Principle

Enzyme activity was measured by method (Farrukh,et al., 2012). The assay quantifies the amount of N-acetyl-D- glucosamine relased from hyaloronic acid by the enzyme .

### Partial Purification of Hyaluronidase by Ion Exchange Chromatography

15 samples of frozen seminal fluid were taken and the supernatant from the sediment by centrifugation cooled at 700 xg from 15 minutes according to the method (Mohamed, 2005) with modification .the enzyme activity was measured by the supernatant because frezzing the samples breaks the acrosome membrane and release the enzyme in to the supernatant (Linder *et al* 1971). The protien was then precipitated using acetone where the addition was gradul for an hour at $-4c^0$. After which it was left in the refrigerater for 24 hours at $4c^0$.

### Determination of Molecular Weight by Electrophoresis

The molecular weight of the hyaloronidase enzyme separated from the seminal fluid was determinned from the group of unexplained infrtile patients by applying an SDS-PAGE electophoresis where the protien solution obtained from package (1) concentrated was injected isolated polyethlene glycol and isolated from the process by applying ion excgange chromatography .through this process aprotien bandle was distinguished at adistance of ( 8 cm) cm from the starting point . this bundle was dopted in estimating the molecular wieght of the enzyme and it was found that is approximately equal to (≈ 59 ) kilo dalton by using the molecular weihgt of standard protien.

**Biochemical Parameter in Seminal Plasma**

Seminal plasma cholesterol was determined by using kit manufactured by manufactured by Biolabo (France) (Burits et al, 2012). Seminal plasma albumin was determine by using kit manufactured by Biolabo (France) . Seminal plasma SH group wss determined by using method (Marder et al., 1994) .TAC measured by (DPPH) methods (Okunade & Wunnava, 2002). Done estimation of element Zn , Mn, Se by uses atomic absorbation (Farrukh,et al 2012).

## Results and Discussion

The results of this study indicated that there were many significant changes in the level of biochemical variables for the group of infertile men compared to the control group through the stages of enzyme separation and purification shown in Table No. (1).

Table 1. Experimental and control group comparison

| Parametars | Fertile(50) | Infertile(150) | p-value |
|---|---|---|---|
| Total cholesterol (mg/dl) | 21.63±12.25 | 33.58±15.78 | 0.01 |
| Albumin (mg/dl) | 0.66±0.18 | 1.06±0.62 | 0.01 |
| Mn (µg/ml( | 70.62±16.85 | 42.05±12.02 | 0.003 |
| Zn (µg/ml) | 274.01±90.23 | 95.44±58.75 | 0.014 |
| Thiol group (µmol/L) | 14.43±3.87 | 6.78±0.93 | 0.00 |
| TAC (% ) | 12.16±0.66 | 1.85±0.42 | 0.00 |

**1-Total Cholestrol**

In this study, it was found that there was a significant increase in the level of cholesterol in the semen plasma of people with unexplained infertility compared with the group of healthy people without infertility and the reason may be due to this To the occurrence of insulin resistance in men who suffer from infertility and this occurs most often in men who suffer from obesity and thus increase the chances of unexplained sterility (Ouvrier et al., 2011). This increase leads to testicular damage and impaired reproductive processes due to the large formation of free radicals and significantly increased oxidative stress (Pushpendra and Jain 2015).

**2-Albumin**

In this study, it was observed that there was a significant increase in the concentration of albumin in the semen plasma of infertile subjects compared with healthy subjects, and the reason may be due to that patients with unexplained infertility have high levels of reactive oxygen species (ROS) which greatly affect the increase in the albumin level in the semen plasma and this greatly affects the sperm motility and vitality (Elzanaty et al., 2007), which increases the symptoms of unexplained infertility and the effect on fertility in infected men(Rodrigues *et al* 2013). As well as proteins increase the viscosity of the semen, which negatively affects the movement of sperm (Harchegani et al., 2019).

**3- Trace Elements**

In this study, it was observed that there was a significant decrease in the trace mineral elements that were measured in the semen plasma, which included zinc and manganese, in men suffering from unexplained infertility compared to healthy men, and this may be due to the role of zinc as an antioxidant, especially the types of reactive oxygen, as the decrease in zinc leads to significant damage resulting from oxidative stress, which greatly affects the quality of the semen and the mobility of the sperm (Lee, 2018). The cause of zinc deficiency may be a lack of zinc intake through food or a dietary interference that prevents the body from absorbing zinc (Powell, 2002). Also, many recent studies have proven that zinc affects the level of white blood cells, as the more of these cells, the higher the level of inflammation and work to reduce the level of zinc (Kerns et al., 2018)

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Manganese plays an important role in improving fertility properties in men through its action to regulate the role of many reproductive hormones, as it, in association with zinc, works to stabilize chromatin and the sperm membrane as well as enhance the mechanical and kinetic properties of sperm (Shquirat et al, 2013). The reason for the significant decrease in manganese in men who suffer from unexplained infertility may be due to a defect in the function of the prostate gland, which leads to a lack of manganese formation and secretion, as many studies have confirmed the role of the prostate gland in regulating the necessary amount of this element to determine the movement of sperm (Barber *et* al., 2005).

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**4-Thiol Group**

In this study, a significant decrease in the thiol group was observed in men suffering from unexplained infertility compared to the control group, and the reason may be due to that the thiol group is considered one of the groups very sensitive to increase the oxidation processes and therefore large quantities of it are consumed to reduce the negative impact of oxidative processes that are in normal people within the normal level and therefore there is a decrease in the level of the thiol group in sterile men (Piomboni et al., 2012). Also, the reason may be due to an increase in oxidation processes leading to an effect on the protein structure and cellular function, especially when the oxidized groups correlate with the thiol group of the amino acid cysteine, which leads to a significant increase in the use of the thiol group and thus a decrease in the level (Gong et al., 2012) as well as some studies noted the presence of (ATPase) (Ca2 + -ATPase) Within the seminal plasma content, which indicates that oxidative processes greatly affect the specialized enzymes and thus reduce the level of thiol groups (Cabrillana et al., 2016).

**5-TAC**

It was observed during this study that there was a significant decrease in the amount of non-enzymatic antioxidants (31) when comparing infertile men with a group of healthy men, and the reason for this may be due to the fact that men with unexplained infertility have a very high percentage of free radicals, which leads to a significant decrease in the level of non-enzymatic antioxidants, which are considered to scavenge free radicals and work to reduce their formation (Agarwal & Sekhon, 2010) as the increase in free radicals leads to a decrease in the susceptibility of sperm Perhaps fertilization, which leads to the emergence of symptoms of unexplained sterility, where the effect is directly on the membranes (Subramanian *et al* ,2018).

**6-Hyaluronic Enzyme**

The results of this study showed the presence of significant biological changes in the level and activity of the hyalurinase enzyme when comparing the group of patients with the control group, and thus the role of the enzyme is very clear through the occurrence of cases of unexplained infertility in men who suffer from low activity of this enzyme in the plasma of semen and confirms the significant role This enzyme and the results of the purification process are shown in Table No (2).

Table 2. The stages of purification of the hyaluronase enzyme

| Step | Volum | Protein con. | Activity | T.P | T.A | Spicific Activity | Flod | Recovery Ratio |
|---|---|---|---|---|---|---|---|---|
| crude | 21 | 5.04 | 42.1 | 105.8 | 884.1 | 8.35 | 1 | 100% |
| Filtrate | 18.5 | 3.91 | 41.98 | 72.44 | 776.73 | 10.72 | 1.28 | 87.86% |
| acetone precipitate | 9.5 | 5.18 | 73.08 | 49.2 | 694.29 | 14.11 | 1.69 | 78.53% |
| DEAE-cellulose | 25 | 0.35 | 21.59 | 8.75 | 539.74 | 61.68 | 7.38 | 61.04% |
| Sephadex-G100 | 20 | 0.113 | 24.4 | 2.27 | 487.94 | 214.95 | 25.74 | 55.74% |

**Gel Filtration Chromatography**

The gel filtration technique was used to separate the protein bundles emerging from the ion exchange technology, where a separation column (60cm*2.5cm) containing a gel of the type (Sephadex G-100) was used. It is noted that there are two protein peaks the enzyme activity is at the second peak as shown in Figure No. (1).

**Molecular Weight by Electrophoresis**

The approximate molecular weight of hyalurinase was estimated using electro-migration technique SDS-PAGE electrophoresis Where the results showed the presence of a protein bundle close to the protein bundle of the standard substance with a molecular weight (58 KDa) As shown in Figure No. (2), and through the use of the standard curve between the value of (Rf) and the value of (log Mw), the approximate molecular weight of the enzyme was calculated, which is within (59 Kda).

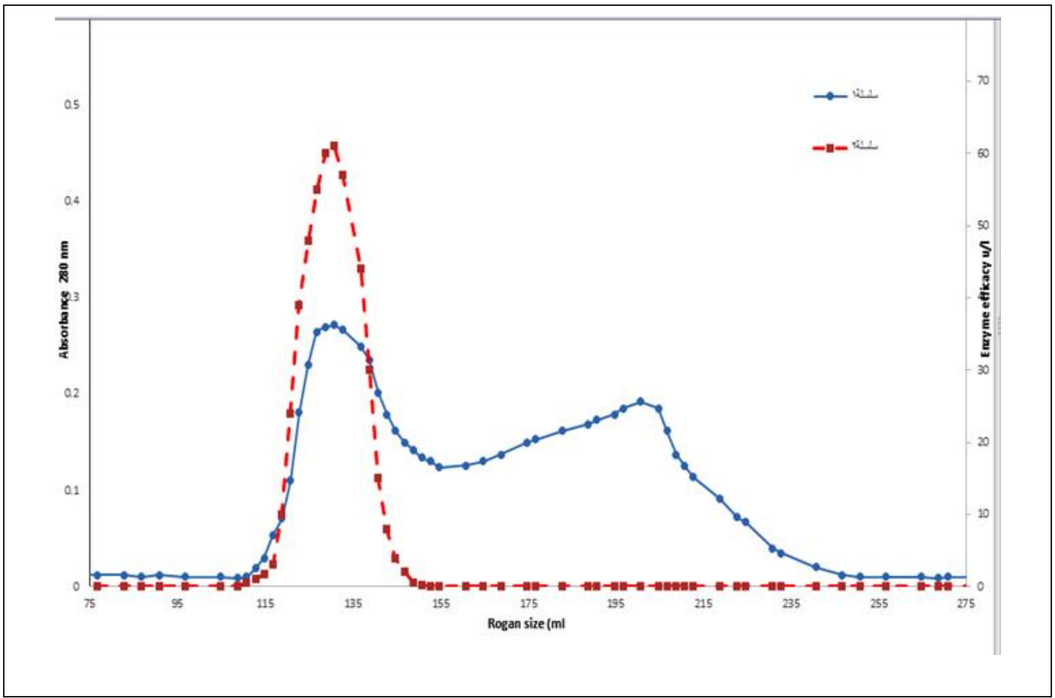


Figure 1. Profile shows the hyalorondase protein bundles generated from separating column (Sephadex G-100 ) of human seme.

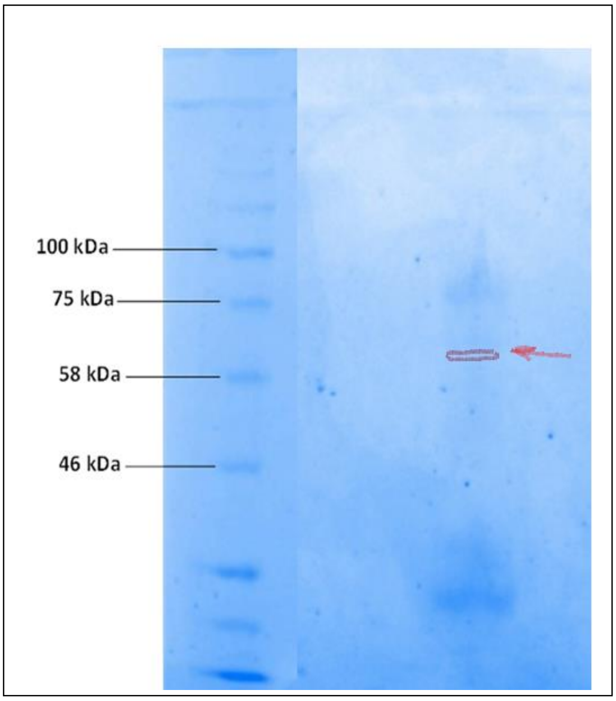


Figure 2. Separated protein bundles on a gel using SDS-PAGE electrophoresis

## Conclusion

The results of the study showed the presence of significant effects of many biochemical variables on the occurrence of unexplained infertility through the role of these variables during various metabolic processes, especially lipid metabolism.

## Recommendations

The study recommends examining the activity of hyalurinase enzyme in patients with unexplained infertility and measuring a number of biochemical variables to find out the reasons that may lead to infertility in men without a clear organic cause

## Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

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## References

Agarwal, A. & Sekhon, L.H., (2010). The role of antioxidant therapy in the treatment of male infertility. *Human Fertility*, *13*(4), pp.217-225.

Asadpour, R., (2012). Relationship between mineral composition of seminal plasma and semen quality in various ram breeds. *Acta Scientiae Veterinariae*, *40*(2), pp.1-8.

Babakhanzadeh, E., Nazari, M., Ghasemifar, S. & Khodadadian, A., (2020). Some of the factors involved in male infertility: A prospective review. *International Journal of General Medicine*, *13*, p.29.

Barber, S.J., Parker, H.M. & McDaniel, C.D., (2005). Broiler breeder semen quality as affected by trace minerals in vitro. *Poultry Science*, *84*(1), pp.100-105.

Bhandari, U., Sharma, J.N. & Zafar, R., (1998). The protective action of ethanolic ginger (Zingiber officinale) extract in cholesterol fed rabbits. *Journal of Ethnopharmacology*, *61*(2), pp.167-171.

Bhandari, U., Sharma, J.N. & Zafar, R., (1998). The protective action of ethanolic ginger (Zingiber officinale) extract in cholesterol fed rabbits. *Journal of Ethnopharmacology*, *61*(2), pp.167-171.

Cabrillana, M.E., Uribe, P., Villegas, J.V., Álvarez, J., Sánchez, R. & Fornés, M.W., (2016). Thiol oxidation by nitrosative stress: Cellular localization in human spermatozoa. *Systems Biology in Reproductive Medicine*, *62*(5), pp.325-334.

De Lazari, F.L., Sontag, E.R., Schneider, A., Moura, A.A., Vasconcelos, F.R., Nagano, C.S., Mattos, R.C., Jobim, M.I.M. & Bustamante-Filho, I.C., (2019). Seminal plasma proteins and their relationship with sperm motility and morphology in boars. *Andrologia*, *51*(4), p.e13222.

El-Beshbishy, H.A., Aly, H.A. & El-Shafey, M., (2013). Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. *Toxicology and Industrial Health*, *29*(10), pp.875-887.

Elzanaty, S., Erenpreiss, J. & Becker, C., (2007). Seminal plasma albumin: Origin and relation to the male reproductive parameters. *Andrologia*, *39*(2), pp.60-65.

Farrukh, M.A., Tan, P. & Adnan, R., (2012). Influence of reaction parameters on the synthesis of surfactant-assisted tin oxide nanoparticles. *Turkish Journal of Chemistry*, *36*(2), pp.303-314.

Farrukh, M.A., Tan, P. & Adnan, R., (2012). Influence of reaction parameters on the synthesis of surfactant-assisted tin oxide nanoparticles. *Turkish Journal of Chemistry*, *36*(2), pp.303-314.

Gong, S., Gabriel, M.C.S., Zini, A., Chan, P. & O'Flaherty, C., (2012). Low amounts and high thiol oxidation of peroxiredoxins in spermatozoa from infertile men. *Journal of Andrology*, *33*(6), pp.1342-1351.

Harchegani, A.B., Rahmani, H., Tahmasbpour, E. & Shahriary, A., (2019). Hyperviscous semen causes poor sperm quality and male infertility through induction of oxidative stress. *Current Urology, 13*(1), pp.1-6.

Jung, H., (2020). Response to letter: Comments on. *Archives of Plastic Surgery, 47*(6), pp.628-628.

Juyena, N.S. & Stelletta, C., (2012). Seminal plasma: an essential attribute to spermatozoa. *Journal of Andrology*, *33*(4), pp.536-551.

Kerns, K., Zigo, M. & Sutovsky, P., (2018). Zinc: A necessary ion for mammalian sperm fertilization competency. *International Journal of Molecular Sciences, 19*(12), p.4097.

Köster, S., Herres, N., Rey, M. & Reichelt, K., (1986). A technique to improve the epitaxial growth of some fcc and bcc metals on rock salt. *Journal of Applied Physics, 59*(1), pp.278-280.

Lee, S.R., (2018). Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. *Oxidative Medicine and Cellular Longevity*, Article ID 9156285, https://doi.org/10.1155/2018/9156285

Linder, L., Nord, C.E. & Söder, P.Ö., (1971). Separation of hyaluronidase from a strain of anaerobic corynebacteria. *European Journal of Oral Sciences, 79*(4), pp.528-532.

Marder, S.R., Cheng, L.T., Tiemann, B.G., Friedli, A.C., Blanchard-Desce, M., Perry, J.W. & Skindhøj, J., (1994). Large first hyperpolarizabilities in push-pull polyenes by tuning of the bond length alternation and aromaticity. *Science, 263*(5146), pp.511-514.

Mohamed, S.A., (2005). Hyaluronidase isoforms from developing embryos of the camel tick Hyalomma dromedarii. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 142*(2), pp.164- 171.

Okunade, A.A. & Murthy, V.N., (2002). Technology as a 'major driver'of health care costs: a cointegration analysis of the Newhouse conjecture. *Journal of Health Economics, 21*(1), pp.147-159.

Okunade, A.A. & Wunnava, P.V., (2002). Availability of health insurance and gender differences in" job-lock" behavior: evidence from NLSY. *Journal of Forensic Economics*, *15*(2), pp.195-204.

Ouvrier, A., Alves, G., Damon-Soubeyrand, C., Marceau, G., Cadet, R., Janny, L., Brugnon, F., Kocer, A., Pommier, A., Lobaccaro, J.M.A. & Drevet, J.R., (2011). Dietary cholesterol- induced post-testicular infertility. *PloS One, 6*(11), p.e26966.

Park, H.Y., Kim, T.S., Lee, S.J., Choi, D.G. & Kang, M.H., (2019). A clinical observation of endocrine adrenal tumors. *Journal of Korean Endocrine Society, 9*(3), pp.228-238.

Piomboni, P., Stendardi, A., Gambera, L., Tatone, C., Coppola, L., De Leo, V. & Focarelli, R., (2012). Protein modification as oxidative stress marker in normal and pathological human seminal plasma. *Redox Report*, *17*(5), pp.227-232.

Powell, S.R., (2000). The antioxidant properties of zinc. *The Journal of Nutrition*, *130*(5), pp.1447S-1454S.

Pushpendra, A. & Jain, G.C., (2015). Hyper-lipidemia and male fertility: A critical review of literature. *Andrology (Los Angel), 4*(2).

Shquirat, W.D., Al-Daghistani, H.I., Hamad, A.R., Abdel-Dayem, M. & Al-Swaifi, M., (2013). Zinc, manganese and magnesium in seminal fluid and their relationship to male infertility in Jordan. *International Journal of Pharmacy and Medical Sciences, 3*(1), pp.01-10.

Subramanian, V., Ravichandran, A., Thiagarajan, N., Govindarajan, M., Dhandayuthapani, S. & Suresh, S., (2018). Seminal reactive oxygen species and total antioxidant capacity: Correlations with sperm parameters and impact on male infertility. *Clinical and Experimental Reproductive Medicine, 45*(2), p.88.

Tvrdá, E., Sikeli, P., Lukáčová, J., Massányi, P. & Lukáč, N., (2021). Mineral nutrients and male fertility. *Journal of Microbiology, Biotechnology and Food Sciences, 3*(1), pp.1-14.

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