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Assessing fertility health: A comparative analysis of serum and seminal plasma levels of key biomarkers in fertile and infertile males

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ABSTRACT

Although the exact procedure of male sterility is unclear, it is a rapidly developing restorative study. Half of infertile couples worldwide suffer from male reproductive failure. The World Health Organization's recommendation for standardized sperm testing will improve analytical accuracy by addressing bias in sperm quality assessment. As a result, little is understood about the biochemical elements found in seminal serum and plasma, where they come from, and how they work physiologically in the male reproductive system. Understanding levels of these pivotal biomarkers in serum and seminal plasma may be a valuable insight into the factors that decide male fertility and help aid in the development of targeted diagnostics and treatment strategies. Sperm motility in 26 fertile men and 50 infertile men was studied in this research about serum and semen levels of several components. Extensive investigation on semen parameters and their relationship with blood biochemical parameters revealed that fertile individuals exhibit normative values in semen volume, concentration, motility, and morphology along with normal plasma concentrations of essential elements like calcium, magnesium, zinc, iron, and fructose, indicating favorable nutritional and metabolic conditions for reproductive health. Conversely, deviations from these normative levels in infertile individuals suggest impaired sperm health and function. The complete investigation's statistically significant data were validated by ANOVA analysis. The results indicated that seminal plasma magnesium remained substantial ($P<0.05$) and that serum magnesium and calcium levels remained meaningly advanced in the fertile collection related with the infertile cluster. Reasons for male infertility will soon be discovered and addressed thanks to technological breakthroughs and creative thinking; therefore, the introduction of noninvasive and diagnostic biomarkers is essential for the near future of treatments.

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INTRODUCTION

It has been revealed that infertility is very common throughout the world. Infertility, according to the World Health Organization (WHO), is the incapability to pregnant after at least a year of sexual contact without protection (Assidi, 2022). According to some reports, between 30 and 55 percent of couples who are infertile have a male component that contributes significantly to their infertility (Babakhanzadeh *et al.*, 2020). There is a clear disparity among these sub-infertile males who are classified as having undiagnosed male infertility, even with the advancements in andrology diagnostic procedures. Male infertility is frequently caused by sperm dysfunction, while there are other potential causes as well (Hamada *et al.*, 2013). Gene mutations, viral disorders, aneuploidies, varicocele, radiation, obstruction of the ejaculatory ducts, chemotherapy, and impotence are the reasons for infertility in men. Due to its complexity, infertility might (Krausz *et al.*, 2022; Panner Selvam *et al.*, 2021). A variety of characteristics are common with male infertility, which includes oligospermia, asthenozoospermia, and teratozoospermia (Milachich and Dyulgerova-Nikolova, 2020). There has been a belief that sperm structure, the number of sperm movements, and DNA integrity are the lone factors contributing to effectual fertilization (Oehninger and Kruger, 2021. Regular investigation of semen, however, is not a consistent method of determining infertility, mainly in those infertile people who ejaculate with apparently suitable characteristics called idiopathic infertility.

Trace elements are crucial for preserving human health (Oehninger and Kruger, 2021; Prasad, 2014). Human semen contains high concentrations of trace elements, such as calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), both in bound and free (ionic) forms. These biological components are crucial in influencing a variety of semen characteristics. Numerous investigations have demonstrated the importance of trace elements in male fertility (Skalnaya and Skalny, 2018; Fallah *et al.*, 2018).

The metabolism and motility of spermatozoa depend on fructose. Spermatozoa get their energy from fructose. The seminal vesicles themselves generate it, while the ductus deferens ampulla also contributes in part. The seminal fructose content has been utilized to assess male accessory gland inflammation and obstructive azoospermia. Numerous authors (Prashanth *et al.*, 2015; Trang *et al.*, 2018) examined how fructose levels in seminal plasma affected sperm and overall density. It has been shown that certain mammals have higher levels of zinc in their semen, and that zinc is essential for spermatogenesis (Toragall *et al.*, 2019; Kumar and Singh, 2016). Male infertility appears to be positively correlated with elevated levels of particular metal ions in blood plasma (Morabbi and Karimian, 2024) or semen (Shquirat *et al.*, 2013; Maciejewski *et al.*, 2022). Sperm metabolism, survival, function, and passage through the female genital canal all depend on seminal plasma. Seminal plasma includes trace elements needed for several vital enzymes because the cations there create an osmotic balance. Consequently, determining male reproductive disorders and evaluating fertility requires the use of seminal plasma biochemical analysis (Chao *et al.*, 2023; Drabovich *et al.*, 2014). There is evidence that changes in the amounts of Ca, Mg, Zn, and Fe in seminal plasma are associated with male infertility. According to extensive evidence, calcium is implicated differently in sperm motility at different stages of sperm maturation. It is also reported to cause the acrosome reaction of mammalian spermatozoa. Semen's ions improve its glycolysis and motility. Iron, calcium, and magnesium levels in seminal plasma have strong positive associations, suggesting that these elements are necessary for the acrosomal response, capacitation, and appropriate spermatozoa maturation (Bieniek *et al.*, 2016; Sengupta *et al.*, 2020).

The purpose of the present work is to determine the scope of these constituents which are essential for proper spermatogenesis and if there is an interference in men's fertility with serum and seminal plasma

content of fructose, zinc, magnesium, calcium, and iron. This paper studies the correlation between fertile and infertile male sperm motility by comparing the serum and seminal plasma fructose, glucose, calcium, zinc, and magnesium contents of 76 men (26 fertile and 50 infertile).

MATERIALS AND METHODS

The study was directed after getting approval from the Research Committee of the Institutional Ethical Commission of Chennai Fertility Centre and Research Institute (CFCRI/IHEC/2023/001).

Subject selection

The study subjects included 50 men attending a fertility clinic, Chennai Fertility Centre and Research Institute, Chennai, India with confirmed infertility, and the control group consisted of 26 men with confirmed fertility. The results of the semen analysis test revealed details regarding the research participants' fertility. Of the 76 men, 26 men were identified as fertile, 17 men were identified with TERATO (Teratospermia) condition, 19 with OAT (Oligo Astheno Teratozoospermia) condition, and 14 men with Severe-OAT (Severe Oligo Astheno Teratozoospermia) condition. Before the samples (semen, serum) were collected, each subject gave their informed consent. An advertisement was used to recruit the control group. Every participant was given a questionnaire. It contains data on medical history, occupational exposure, medication use, lifestyle choices (diet, alcohol, and tobacco), and sociodemographic traits (du Plessis *et al.*, 2015).

Inclusive criteria

First, the study group consisted of all the guys who came in for infertility assessments. Second, the control group was made up of healthy volunteers who had never experienced infertility issues and whose partners conceived naturally after a year of consistent, unprotected sexual activity.

Male volunteers with proven fertility, for example, if either the wife is currently pregnant in the 1st trimester or recently delivered a child less than 6 months from the date of enrollment in the study, provided these are natural pregnancies without any medical interventions, were chosen as the control group (du Plessis *et al.*, 2015).

Exclusive criteria

Age groups below 22 years and Participants undergone radiotherapy and chemotherapy related to malignancy were excluded (Skoracka *et al.*, 2020).

Collection of semen samples and evaluation of physical and morphological characteristics

Following two to seven days of abstinence, sperm models were composed by masturbating in a germ-free vessel. Before being inspected, the samplings were permitted to liquefy for thirty minutes at room heat. Through microscopic analysis, the number of sperm, the proportion of motile sperm, and the sperm with normal morphology were all determined objectively. The WHO Laboratory Manual for the Examination and Processing of Human Semen (World Health Organization, 2021) evaluated these parameters (Wang *et al.*, 2022). The four subject groups listed below were created by WHO regulations: Table 1 shows Terminologies associated with male factor fertility.

Estimation of biochemical markers in serum and seminal plasma (Feng *et al.*, 2015; Talluri *et al.*, 2017; Płaczkowska *et al.*, 2024; Kataria *et al.*, 2021)

Estimation of biochemical markers in serum

Blood samples were collected from the study participants, with approximately 5 ml of blood drawn from a hand vein. All samples were sent to the Biochemistry Department at Chennai Fertility Centre for analysis. After being put in gel tubes, the blood was left to coagulate.

Following clotting, the models were centrifugated for ten minutes at 2000 rpm to extract the fluid. Serum was used right away to identify the factors in this investigation. The Erba Chem 5X Semi-Automated

Biochemistry Analyzer® spectrophotometric analyzer was used to calculate the biochemical points of serum and pivotal plasm.

Seminal plasm biological marker estimation

Following evaluation of the physical and morphological criteria, the semen samples were centrifugated for ten minutes at 1500 rpm to eliminate cellular components. The supernatant seminal plasma remained then meticulously gathered for the experiment to guarantee that the particle at the bottom stayed undisturbed. Every parameter was measured in seminal plasma and serum.

Table 1. Terminologies associated with male factor fertility (Wang *et al*., 2022)

Term	Definition
Normozoospermia	Semen sample with normal characteristics including sperm concentration of 16 million/ml or more, Sperm motility (progressive+ nonprogressive) being< 42%, normal sperm morphology <4%
Oligoasthenoteratozoospermia (OAT)	Decreased concentration (<16 million/ml), decreased motility (< 42%), decreased percentage of normal sperm morphology <4%
Severe oligoasthenoteratozoospermia (SOAT)	Low sperm count, poor motility, poor morphology
Teratospermia	Decreased percentage of normal sperm morphology <4%

Estimation of fructose

It had then a 200 µL sample mixed with 800 µL of distilled water and determined the amount of fructose. After proper mixing of the mixture, it is kept at room temperature ranging from 16–25°C for 10 minutes. At 340 nm, the absorbance was measured against a blank following the 10-minute incubation time. Fructose concentration stayed resolute using a Biosystems S.A. kit.

Estimation of zinc

Using a colorimetric technique (Diaspertz kit, Austria) at a wavelength of 560 nm, the zinc concentrations in serum and seminal plasma were determined. Zinc and 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)-phenol combine to create a red chelate complex. The total zinc concentration in the sample determines how much the absorbance of this complex increases. Milligrams per liter was the unit of measurement used.

Estimation of calcium

A colorimetry method (Diaspertz kit, Austria) operating at 650 nm and 22–25°C was used to calculate the calcium concentrations in serum and influential clot. at a pH of neutral. Arsenazo III and calcium combine to generate a blue-colored compound. The content of calcium has a direct correlation with the intensity of the color that is produced as a result. Magnesium interference is removed by adding 8-hydroxyquinoline-5-sulfonic acid. The findings were expressed in mg/dL.

Estimation of magnesium

Magnesium absorptions in blood serum and formative plasm were measured using a colorimetric method (Diaspertz kit, Austria) operated at 520 nm and a temperature of 22-25°C, with concentrations expressed in milligrams per deciliter (mg/dL). In an alkaline solution, magnesium ions and xylidyl blue combine to generate a colorful complex. The attention of magnesium in the sample determines how intense the purple color is. EDTA is used to prevent calcium interference by complexing with calcium irons.

Estimation of iron

The absorption of iron in the serum and pivotal plasm photometrically was determined in the Diaspertz kit. Ferene with ascorbic acid forms a blue compound with ferric iron after being reduced from iron bound to transferrin. This reduces ferric iron into ferric iron and the attendance of ascorbic acid. The intensity of coloration read spectrophotometrically was directly proportional to absorbance at 595 nm, and the results obtained were expressed in micrograms per decilitre (µg/dL).

Transferrin $(Fe^{3+})_2$ $\xrightarrow{\text{Asc. Acid, Buffer}}$ 2 Fe^{2+} + Transferrin

Fe^{2+} + 3 Ferene $\rightarrow$ Ferrous Ferene (Blue complex)

Serum and seminal plasma levels of fructose, zinc, calcium, magnesium, and iron were statistically analyzed

The results of estimation of various biochemical parameters were performed thrice and mean values were recorded along with their standard deviation values to plot the corresponding graphs. The statistical interpretations were done using the ANOVA analysis in SPSS software version 23.

RESULTS AND DISCUSSION

Evaluation of physical and morphological appearances

Sperm count, motile semen percentage, and sperm with normal morphology were all objectively assessed under a microscope. As stated in section 2.4, the sperm count and motile sperm percentage were assessed. The different parameters that were evaluated are shown in Table 2.

Biochemical marker estimation in serum and seminal plasma, along with statistical evaluations

The estimation of the mentioned biological strictures in blood fluid and seminal fluid was done by section 2.5. The statistical interpretations were done using ANOVA in SPSS software version 23 as per section 2.6 and the results were recorded and tabulated (Table 3, Fig. 1 and 2). Statistical significance with an appropriate p-value ($p<0.01$) with a 95% confidence level was observed for all assays.

An estimated 50% of all reproductive issues are thought to be caused by male factor infertility. These issues either affect sperm motility after production or interfere with the sperm manufacturing process. Population-based research is necessary to comprehend the leanings in male generative illnesses and the ecological issues affecting male procreative strength. Trace elements can affect male fertility in different ways and are necessary for both sperm production and quality. Maintaining physiological homeostasis requires a variety of trace amounts of metals; it is well known that high or low quantities of these elements can be harmful and result in insufficiency symptoms. Many studies conducted in the past few decades have established a decline in sperm attentiveness, motility, and usual geomorphology over time, along with an growth in male infertility and the occurrence of certain disorders related to the masculine reproductive scheme, all of which point to a decline in male fecundity (Hamad *et al.*, 2014; Abd Elhady *et al.*, 2021; De Jonge and Barratt, 2019).

In fertile individuals, the mean semen volume falls within the normal range of 3.2±1.26 ml, with a concentration of 31.9±11.78 million sperm per milliliter. Motility percentages are notably higher in fertile individuals, with rapid progressive motility at 23.5±4.74%, slow motility at 26.5±5.79%, and non-progressive motility at 11.5±3.37%. Abnormal morphology percentage is also relatively low at 6.1±1.73%, indicating a higher proportion of morphologically normal sperm. Across different types of infertility (Severe-OAT, OAT, and Terato), there are notable reductions in semen volume, concentration, and motility percentages compared to fertile individuals. For instance, individuals with Severe-OAT exhibit significantly lower semen volume (2.35±0.82 ml) and concentration (4.28±3.99 million/ml) along with reduced motility percentages across all categories. Similarly, individuals with OAT and Terato also demonstrate diminished semen parameters indicative of compromised fertility. Abnormal morphology percentages vary slightly, with some cases showing higher values, indicating increased sperm abnormalities in infertile individuals. Variations in semen properties, such as volume, concentration, motility, and shape, indicate that infertile people may have problems with sperm production and function (Mahdi, 2021). These findings underscore the importance of assessing multiple semen parameters to comprehensively evaluate fertility status.

Table 2. Evaluation of various physical parameters in semen.

Physical parameters	Fertile						Infertile						Sig.
	NORMO			SOAT			OAT			TERATO			
	Mean	Range	CL	Mean	Range	CL	Mean	Range	CL	Mean	Range	CL	
Semen volume	3.2± 1.26	1.5 to 5	0.9	2.35± 0.82	1 to 3.5	0.58	2.4± 0.78	1 to 4	0.43	1.96± 0.74	0.5 to 3	0.38	**
Sperm conc.	31.9± 11.78	15 to 50	8.43	4.28± 3.99	1 to 13	3.69	8.3± 2.84	4 to 13	1.57	21.88± 4.38	15 to 32	2.25	**
Sperm motility %	61.5± 7.47	50 to 70	5.34	34.28± 5.34	30 to 40	4.94	34.33± 9.04	20 to 50	5	53.53± 4.92	50 to 60	2.53	**
Rapid progressive %	23.5± 4.74	20 to 30	3.39	6.43± 3.78	0 to 10	3.49	8± 5.28	0 to 20	2.92	18.23± 5.28	10 to 30	2.72	**
Slow motility %	26.5± 5.79	20 to 35	4.15	17.14± 6.98	10 to 30	6.46	15± 5.67	10 to 25	3.14	24.12± 5.07	15 to 30	2.61	**
Non progressive %	11.5± 3.37	10 to 20	2.41	10.71± 4.49	5 to 20	4.16	15.33± 12.88	10 to 60	7.13	11.18± 3.32	10 to 20	1.71	-
Abnormal sperm morphology %	6.1± 1.73	4 to 9	1.24	1	1 to 1	0	1	1	0	1.41± 0.51	1 to 2	0.26	**

Sperm capacity, sperm concentration, motility, and morphological characters were estimated in normal (in fertile men) and diseased conditions (in infertile men), and the variations observed were noted ($p<0.01$). ** P-values less than 0.05 were measured as statistically important. NORMO – Normozoospermia; SOAT – Severe Oligo Astheno Teratozoospermia; OAT - Oligo Astheno Teratozoospermia; TERATO – Teratospermia.

Table 3. Evaluation of various biochemical markers in semen and blood serum

Parameters groups	Fluid	Group category	Sample number	Mean	Std. deviation	Significance
Fructose	Semen	SOAT	14	421.45	140.4574162	No significance
		OAT	19	328.9789	128.4428908	
		TERATO	17	351.7888	176.3190035	
		Control	26	311.1077	121.1487711	
		Total	76	345.0014	143.0670987	
	Blood	SOAT	14	438.75	65.47205804	No significance
		OAT	19	442.8053	128.8593267	
		TERATO	17	379.5418	197.0978311	
		Control	26	379.8615	101.4974249	
		Total	76	406.3738	131.8646948	
Zinc	Semen	SOAT	14	630.8071	193.3001113	No Significance
		OAT	19	687.1947	198.2204902	
		TERATO	17	746.3353	198.2353618	
		Control	26	602.4846	244.2964456	
		Total	76	661.0566	217.6352988	
	Blood	SOAT	14	238.8043	149.8512943	No significance
		OAT	19	206.1095	254.6309649	
		TERATO	17	223.0312	188.8298665	
		Control	26	205.1715	129.0148126	
		Total	76	215.5964	181.0572254	
Iron	Semen	SOAT	14	57.39214	24.05979832	No significance
		OAT	19	56.02105	23.66456866	
		TERATO	17	62.90941	14.91786826	
		Control	26	90.27962	127.0401683	
		Total	76	69.53447	76.77809152	
	Blood	SOAT	14	57.49571	9.400108604	No significance
		OAT	19	47.34579	19.16991982	
		TERATO	17	43.52588	16.51841096	
		Control	26	54.66231	25.65133826	
		Total	76	50.86408	20.23869925	
Calcium	Semen	SOAT	14	9.264286	4.894010019	No significance
		OAT	19	8.076316	4.140484688	
		TERATO	17	9.755294	6.514807286	
		Control	26	10.68308	10.57798989	

		Total	76	9.5625	7.459109531	
	Blood	SOAT	14	7.945714	3.708922121	Significant **
		OAT	19	10.50421	1.400889859	
		TERATO	17	9.69	1.779480261	
		Control	26	11.62846	2.429228178	
		Total	76	10.23539	2.689810125	
Magnesium	Semen	SOAT	14	6.894286	4.405813841	Significant**
		OAT	19	7.422632	4.845783783	
		TERATO	17	6.249412	4.71620275	
		Control	26	9.926538	4.366116757	
		Total	76	7.919474	4.731490785	
	Blood	SOAT	14	6.823571	3.025114383	Significant**
		OAT	19	6.413158	3.310605941	
		TERATO	17	4.917059	3.627609	
		Control	26	8.352308	4.493072274	
		Total	76	6.8175	3.928097461	

The presence of cations like Calcium, Magnesium, Zinc, and Iron and sugars like fructose were evaluated in sperm and blood fluid of fertile men and infertile men and the variations observed were noted (p<0.01).

**Significance level (95% confidence level) and P value (p < 0.01)

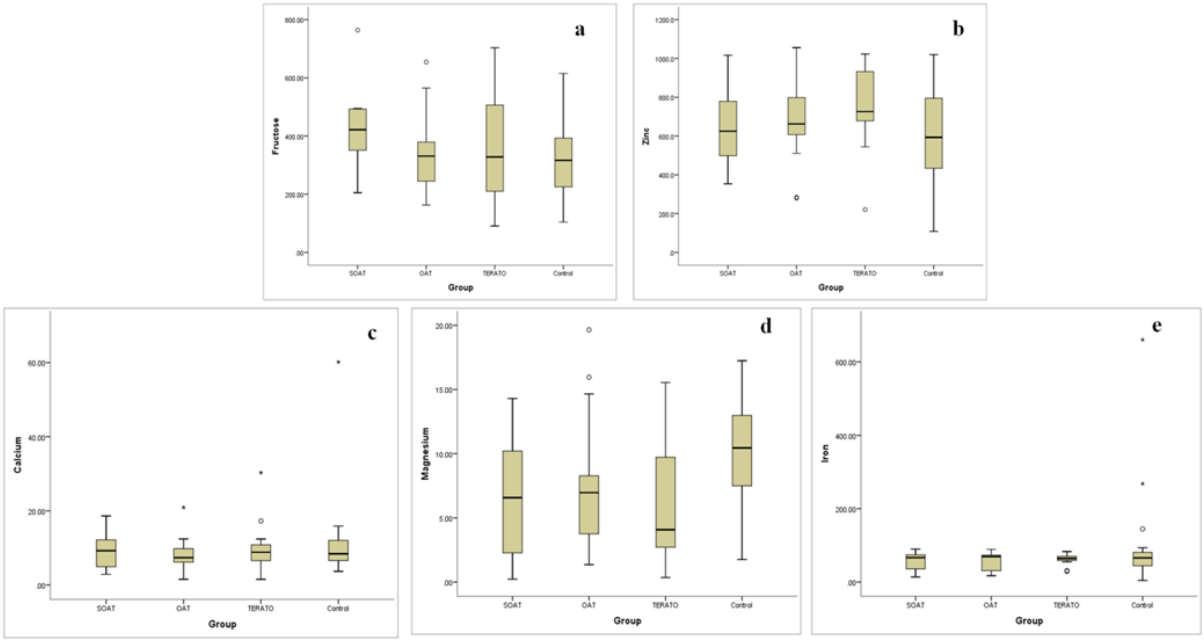

Fig. 1a -e. Evaluation of various biochemical markers in seminal fluid or semen

Presence of cations like Calcium, Magnesium, Zinc and Iron along with fructose was evaluated in sperm of fertile men and infertile men and the graphs were plotted accordingly (p<0.01).

Obesity brought on by elevated blood fructose levels may result in male infertility because it lowers testosterone, sperm volume, entire sperm count, and semen attention. Even though in the present investigation, there is no significant relation shown about blood fructose levels.

Analyzing blood fructose levels control group and Cerato group and control group (379.5 mg/dl) showed the lowest mean value and OAT (442.8 mg/dl) patients exhibited the highest mean value.

It is always imperative to keep the blood fructose levels at their adequate value for the betterment of reproductive health and a higher level of laevulose has been connected to a increase in overweightness, metabolic disorders, and cardiovascular disease (Rizkalla, 2010). These values indicate adequate

nutritional status and metabolic function, which are crucial for supporting reproductive health.

Fructose levels, which are crucial for sperm energy metabolism, show variability across classifications. The control group showed the lowest mean value (311.10 mg/dl) and SOAT patients exhibited the highest mean value (421.25 mg/dl), indicating potential differences in energy metabolism among fertility groups. seminal fructose concentration in patients with infertility SOAT, OAT, and TERATO were significantly higher than in respective control groups.

As put across by Rajalakhshmi *et al.* (1989), and (Gonzales and Villena, 2001), an increase in the concentration of sperm is always coupled with a decrease in seminal plasma fructose concentration because fructose is highly crucial for the energy of sperms. Fructose present in semen forms one of the energy sources of all spermatogenic activities. The higher the semen concentration, vitality, and motility, the less will be the amount of fructose needed. Fructose seminal concentration negatively correlates with sperm volume, concentration, and motility (Trang *et al.*, 2018; Trang *et al.*, 2018). Fertile men use more fructose as an vigor cause for sperm motility and function. The higher concentration of fructose in infertile men further indicates that less utilization of energy by spermatozoa. Therefore, an increased concentration of fructose may be an indicator of the diagnosis of male fertility.

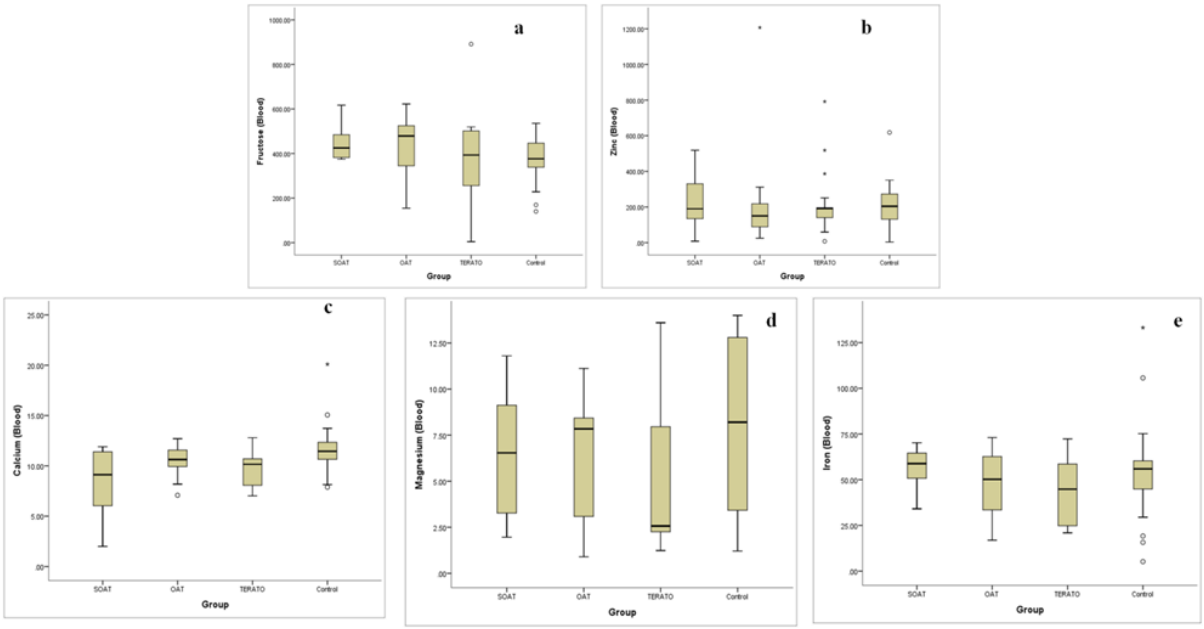

Fig. 2a-e. Evaluation of various biochemical markers in blood serum

The presence of cations like Calcium, Magnesium, Zinc, and Iron along with fructose was evaluated in the blood fluid of fertile men and infertile men, and the graphs were plotted accordingly ($p<0.01$).

Zinc seems to act as a rummager of excess superoxide anion anions produced by damaged sperm and leucocytes in humanoid semen post-ejaculation (Plante *et al.*, 1994; Irvine *et al.*, 1996). Hence, the high zinc concentration in formative plasma appears to provide adequate antioxidant-like protection against the accumulation of excess superoxide anions (Gavella and Lipovac, 1998). Superoxide anions produced by the aberrant spermatozoa attach to zinc in the formative clot and reduce its concentration. Therefore, examining the relationship amid sperm parameters and seminal plasma and blood zinc levels just using infertile individuals may not be instructive. According to Chia *et al.* (2000), there was no important variance in the regular income of the blood zinc level between the male infertility group and the

control group. On the other point, a recent study by Abd Elhady *et al.* (2021) discovered that low blood zinc levels are linked to sperm of poor quality. In our investigation, there was no correlation between blood zinc levels and infertile groups and control groups.

Blood serum zinc levels also showed significant variation between groups, with the CONTROL group having the lowest mean (205.17 mg/dl) and SOAT patients having the highest mean (238.8 mg/dl). The control group's seminal plasma and serum had the lowest zinc stages. According to our research, influencing plasma has greater mean zinc attention than serum. This negative relationship between zinc levels and fertility classification, where higher levels were associated with poorer fertility outcomes, indicates that zinc might be involved in sperm health.

Research on the connection between zinc and male fertility in the population is useless as we know how various conditions, including zinc deficiency, impact the male reproductive system.

There are known positive relationships between seminal plasma zinc, morphology, progressive motility, and sperm concentration (Osadchuk *et al.*, 2021; Kothari and Chaudhari, 2016). A thorough meta-analysis of the relationship between the two variables, carried out by numerous publications, revealed that the seminal zinc concentration of infertile men with asthenozoospermia was considerably lower than that of fertile men (Taravati and Tohidi, 2016). Nevertheless, several studies were unable to show a correlation between seminal zinc levels and semen characteristics or a distinction between fertile men and those who are not (Hashemi *et al.*, 2018; Wong *et al.*, 2001). The men in this study had mean seminal zinc concentrations that were comparable to those in a number of previous studies. Studies looking into the relationship between seminal zinc and typical semen traits like sperm count, motility, and morphology have produced contradictory results. Consequently, there are presently differing views about the relationship between seminal zinc and sperm quality indicators. In addition to finding no positive correlation between serum zinc content and other semen indicators such as volume, total sperm count, sperm concentration, progressive motility, and normal morphology, the study also found significant differences between groups with normal and poorer semen quality. According to the findings of the current study, additional authors also did not find significant differences in fluid zinc levels between the groups with normal and poor sperm quality, or even between the productive and infertile groups (Liu *et al.*, 2020; Saleh *et al.*, 2008). OAT patients had the greatest mean (644.1±225.7) in our study, whereas the control group had the lowest (602.485 ±244.38). This negative relationship between zinc levels and fertility classification suggested that zinc might be involved in sperm health, with higher levels linked to poorer fertility outcomes. Excessive concentration is linked to reduced motility in OAT patients.

Numerous proteins and enzymes, and consequently cellular homeostasis, depend on calcium and magnesium. These factors are therefore tightly controlled, a procedure in which the blood-seminal plasma barricade acts a crucial part. It is unknown, therefore, exactly how these components are moved from the bloodstream into the seminal plasma. Magnesium's role in spermatozoa quality is yet unclear. All processes that rely on intracellular magnesium, including respiration, reproduction, glycolysis, and protein synthesis, are known to be impacted by its depletion (Tielemans *et al.*, 1997). Magnesium levels in blood serum showed significance as well ($p<0.05$), with the control group exhibiting the highest mean (8.3523 ± 1.24 mg/dl) and TERATO patients having the lowest mean (4.971±1.24 mg/dl), suggesting potential systemic influences on magnesium levels in different fertility classifications. Magnesium is vital for sperm health, impacting motility, morphology, and concentration (Onnarakatt *et al.*, n.d.). In our study, it is shown that there is a significant correlation between blood Mg levels in the fertility of men. According to (Prien *et al.*, 1990), men

with reduced sperm motility had significantly lower blood calcium levels, but their total calcium levels were the same as those of men with normal motility. We also discovered a surprising positive association between magnesium and calcium (Fawcett *et al.*, 2000; Wong *et al.*, 2001). Analyzing calcium in blood serum parameters showed significant differences in calcium levels among classifications, with SOAT patients having the lowest mean and the Control group exhibiting the highest. A notable interaction between serum calcium and infertility was found (P <001). Thus, both nutrients play crucial roles in enhancing the reproductive health of males (Skoracka *et al.*, 2020). Regarding male fertility, calcium is indispensable for sperm movement, the production and maturation of sperm (spermatogenesis), the acrosome reaction needed for fertilization, and the regulation of reproductive hormones. Maintaining adequate calcium levels is crucial for overall health and reproductive efficacy. Lower serum calcium levels have been consistently observed in infertile men compared to fertile men. This difference highlights the importance of the estimation of seminal plasma during male fertility evaluation.

Magnesium is another important component for cell activity and is typically found in significant amounts in semen. A cation that is required in almost all enzyme systems is magnesium. Magnesium is a cofactor in over three hundred enzymatic processes, including vigor absorption (ATP) and nucleic acid mixture, and it also modifies some enzyme substrates. Magnesium may be involved in spermatogenesis, namely in the motility of sperm. Moreover, magnesium functions as an intracellular calcium antagonist and is thought to be a marker of seminal vesicle secretions (Wong *et al.*, 2001). Our current study's findings demonstrated that OAT, SOAT, and TERATO patients' infertile seminal plasma magnesium levels had significantly decreased. Because magnesium is a calcium antagonist, the control group had the greatest mean magnesium levels (9.9±4.3 mg/dl), while TERATO patients had the lowest mean (6.2±4.7 mg/dl) (Liang *et al.*, 2016). This knowledge leads us to believe that infertile men's aberrant seminal plasma magnesium levels may disrupt calcium's normal biological activity. Therefore, this study suggests that there may be a significant correlation between infertility and the amount of magnesium in formative plasma.

(Abdul-Rasheed, 2010) discovered that all infertile groups had significantly lower Mg concentrations in seminal plasma as compared to normozoospermic fertile men. Research by (Bassey *et al.*, 2013) found that infertile males' seminal plasma had markedly lower calcium and magnesium levels than fertile ones. No discernible variations in the seminal plasma magnesium levels between the infertile and fertile groups were observed by (Colagar *et al.*, 2009). (Sørensen *et al.*, 1999) looked at whether seminal plasma levels of calcium and magnesium, among other things, have a important impact on a healthy couple's delay to conception. Males from couples with a short time to pregnancy (one month) and men from couples with a long time to pregnancy (10 months) did not exhibit any appreciable differences in the levels of these components. According to the information provided, magnesium acting a crucial role in maintaining oxidative antioxidant balance in the male reproductive tract. This function is somewhat complicated by nature, and it should be taken into account as part of a management strategy for male infertility.

Whole-body metabolism changes can affect testicular function, and micronutrients are thought to be essential for maintaining a healthy metabolism (Giahi *et al.*, 2016; Machen and Sandlow, 2020). For healthy male reproduction and proper spermatogenesis, iron is one vitamin that is required. Iron deficiency and iron deficiency can result in anemia [IDA], a severe public health concern (Tsao *et al.*, 2022). Males must consume a lot of iron since it is essential for preserving the fluidity of ejaculation and the pH of sperm within a useful range (Vanderhout *et al.*, 2021). Furthermore, Sertoli and Leydig cells shield testicular tissue and are great suppliers of ferritin for

growing sperm (Wise *et al.*, 2003). In our present investigation, iron does not show much significance Fe levels in blood serum are also consistent across the groups, with TERATO patients showing the lowest mean (43.5 mg/dl) and SOAT groups showing the highest mean (57.4 mg/dl). By causing lipid peroxidation and ROS, elevated Fe levels may be the cause of poor motility. A pilot investigation by (Soliman *et al.*, 2014) found that in eugonadal males with iron-deficiency IDA, IDA correction was linked to increased levels of blood LH, FSH, and testosterone (Tvrda *et al.*, 2015) as well as a significant improvement in sperm parameters. Gonadotropin hormones are raised when IDA is corrected because it relieves hypoxia (Soliman *et al.*, 2013). These studies show how important iron is for male reproduction, but it is concerning because our study population is less aware of these factors. In summary, our results suggest that iron may act as a mediator of the effects of oxidative damage and be essential for spermatogenesis and male infertility; it is always recommended to measure the amount of iron in serum and seminal plasma when investigating infertility.

Life needs iron. The manufacture of responsive oxygen species would likewise be catalysed by iron. The human body strictly regulates iron intake from food to preserve equilibrium. When these regulatory mechanisms malfunction, serious iron overloading illnesses can result, such as β-thalassemia and hereditary hemochromatosis. Iron is a necessary element; however, iron overload mostly affects the male reproductive system. When excessive iron from iron overload problems is not eliminated, it typically leads to hypogonadotropic hypogonadism, infertility, and sexual dysfunction (Gabrielsen *et al.*, 2018). Iron Deficiency Anemia (IDA), which is a documented iron deficiency, is nevertheless a significant public health concern (Kumar *et al.*, 2022). Malaria, non-specific inflammation, iron and other vitamin shortages, and other hereditary blood abnormalities are some of the most prominent causes of IDA. The burden of IDA worldwide is primarily found in women and children, but numerous studies have also shown an increased incidence in males; the most common causes were found to be poor nutrition and chronic GI blood loss. IDA is estimated to affect two to five percent of all mature males and may hurt their reproductive outcomes (Akhter *et al.*, 2021). In a pilot trial, (Soliman *et al.*, 2017) found that in eugonadal males with iron-deficient IDA, treatment of IDA was associated with a significant improvement in sperm parameters and increased serum levels of LH, FSH, and testosterone (Eroglu *et al.*, 2014). Given that semen iron levels are rather constant across fertility categories in the current investigation, iron may not be a significant determinant of sperm health in this instance either. Our results were in line with a study by (Aydemir *et al.*, 2006), which found no discernible variation in the subfertile male group's serum Fe level. It is generally advised to evaluate the quantity of iron in serum and seminal plasma when studying infertility, as our data imply that iron may be significant for spermatogenesis and male infertility, as well as an intermediary mediator of the effects of oxidative damage.

This study suggests a possible connection amid seminal fructose and zinc levels, and infertility classifications. The significantly higher seminal fructose concentrations in infertile groups, compared to controls, coupled with notable differences in blood fructose levels, imply a latent part for fructose in the pathophysiology of infertility. Additionally, variations in zinc levels across classifications may indicate a connection between zinc and reproductive dysfunction. While iron levels remained consistent across fertility classifications, important plasma magnesium and serum calcium and magnesium levels showed a significant association with fertility status, warranting further investigation into their potential as biomarkers for infertility diagnosis. These findings donate to our consideration of the complex dealings among these biomarkers and infertility, highlighting areas for future research and potential clinical applications.

CONCLUSION

Although numerous lifestyle factors, such as poor eating, are considered to be significant contributors to male reproductive health impairment, the causes of diminishing male fertility remain unexplained. This is even though several proofs consume encouraged multiple experts to transmit out epidemiologic investigations in various nations worldwide. Idiopathic infertility is the term used to describe the more than 30% of male infertility cases that are still unsolved. The reasons for male subfertility and infertility must be better understood to cure infertility and comprehend the regulatory frameworks of male reproductive function. The impact of various biochemical markers in blood serum and semen in together productive and infertile men was compared in the current investigation. However, we must remember that the chemical components of spermatozoa can vary and rely primarily on the number of semen cubicles present in the ejaculate. The results of the study suggest that trace elements can play an important part in infertility toxicity, which cannot be explained. With these components added to an appropriate formula, therapeutic intervention may be helpful and may soon offer a guarantee of modern infertility therapies.

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REFERENCES

Abd Elhady MS, Kandil AH, Albalat WM. 2021. Trace element's role in male infertility: Review article. The Egyptian Journal of Hospital Medicine **85**(2), 3678–3681.

Abd Elhady MS, Kandil AH, Albalat WM. 2021. Trace element's role in male infertility: Review article. The Egyptian Journal of Hospital Medicine **85**(2), 3678–3681.

Abdul-Rasheed OF. 2010. Association between seminal plasma copper and magnesium levels with oxidative stress in Iraqi infertile men. Oman Medical Journal **25**(3), 168.

Akhter MS, Hamali HA, Iqbal J, Mobarki AA, Rashid H, Dobie G, Laghbi OS. 2021. Iron deficiency anemia as a factor in male infertility: Awareness in health college students in the Jazan Region of Saudi Arabia. International Journal of Environmental Research and Public Health **18**(24), 12866.

Assidi M. 2022. Infertility in men: Advances towards a comprehensive and integrative strategy for precision theranostics. Cells **11**(10), 1711.

Aydemir B, Kiziler AR, Onaran I, Alici B, Ozkara H, Akyolcu MC. 2006. Impact of Cu and Fe concentrations on oxidative damage in male infertility. Biological Trace Element Research **112**, 193–203.

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**, 29–41.

Bassey IE, Essien OE, Udoh AE, Imo IU, Effiong IO. 2013. Seminal plasma selenium, calcium, magnesium and zinc levels in infertile men. (Incomplete citation details – please provide journal, volume, pages, and year).

Bieniek JM, Drabovich AP, Lo KC. 2016. Seminal biomarkers for the evaluation of male infertility. Asian Journal of Andrology **18**(3), 426–433.

Chao HH, Zhang Y, Dong PY, Gurunathan S, Zhang XF. 2023. Comprehensive review on the positive and negative effects of various important regulators on male spermatogenesis and fertility. Frontiers in Nutrition **9**, 1063510.

Chia SE, Ong CN, Chua LH, Ho LM, Tay SK. 2000. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. Journal of Andrology **21**(1), 53–57.

Colagar AH, Marzony ET, Chaichi MJ. 2009. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. Nutrition Research **29**(2), 82–88.

De Jonge C, Barratt CL. 2019. The present crisis in male reproductive health: An urgent need for a political, social, and research roadmap. Andrology **7**(6), 762–768.

Drabovich AP, Saraon P, Jarvi K, Diamandis EP. 2014. Seminal plasma as a diagnostic fluid for male reproductive system disorders. Nature Reviews Urology **11**(5), 278–288.

du Plessis SS, Agarwal A, Mohanty G, Van der Linde M. 2015. Oxidative phosphorylation versus glycolysis: What fuel do spermatozoa use?. Asian Journal of Andrology **17**(2), 230–235.

Eroglu M, Sahin S, Durukan B, Ozakpinar OB, Erdinc N, Turkgeldi L, Karateke A. 2014. Blood serum and seminal plasma selenium, total antioxidant capacity and coenzyme Q10 levels in relation to semen parameters in men with idiopathic infertility. Biological Trace Element Research **159**(1), 46–51.

Fallah A, Mohammad-Hasani A, Colagar AH. 2018. 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 & Infertility **19**(2), 69–76.

Fawcett WJ, Haxby EJ, Male DA. 2000. Magnesium: Physiology and pharmacology. Survey of Anesthesiology **44**(2), 97.

Feng RX, Lu JC, Zhang HY, Lü NQ. 2015. A pilot comparative study of 26 biochemical markers in seminal plasma and serum in infertile men. BioMed Research International **2015**(1), 805328.

Gabrielsen JS, Lamb DJ, Lipshultz LI. 2018. Iron and a man's reproductive health: The good, the bad, and the ugly. Current Urology Reports **19**, 1–7.

Gavella M, Lipovac V. 1998. In vitro effect of zinc on oxidative changes in human semen. Andrologia **30**(6), 317–323.

Giahi L, Mohammadmoradi S, Javidan A, Sadeghi MR. 2016. Nutritional modifications in male infertility: A systematic review covering 2 decades. Nutrition Reviews **74**(2), 118–130.

Gonzales GF, Villena A. 2001. True corrected seminal fructose level: A better marker of the function of seminal vesicles in infertile men. International Journal of Andrology **24**(5), 255–260.

Hamad AWR, Al-Daghistani HI, Shquirat WD, Abdel-Dayem M, Al-Swaifi M. 2014. Sodium, potassium, calcium and copper levels in seminal plasma are associated with sperm quality in fertile and infertile men. Biochemical Pharmacology **3**(4), 1–7.

Hamada AJ, Esteves SC, Agarwal A. 2013. A comprehensive review of genetics and genetic testing in azoospermia. Clinics **68**, 39–60.

Hashemi MM, Behnampour N, Nejabat M, Tabandeh A, Ghazi-Moghaddam B, Joshaghani HR. 2018. Impact of seminal plasma trace elements on human sperm motility parameters. Romanian Journal of Internal Medicine **56**(1), 15–20.

Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. 1996. Evidence of deteriorating semen quality in the United Kingdom: Birth cohort study in 577 men in Scotland over 11 years. BMJ **312**(7029), 467–471.

Kataria JUHI, Gill GK, Cojandaeaj L. 2021. Relationship of seminal fructose and serum prolactin levels in infertile men. Asian Journal of Pharmaceutical and Clinical Research **14**(11), 85–87.

Kothari RP, Chaudhari AR. 2016. Zinc levels in seminal fluid in infertile males and its relation with serum free testosterone. Journal of Clinical and Diagnostic Research **10**(5), CC05.

Krausz C, Rosta V, Swerdloff RS, Wang C. 2022. Genetics of male infertility. Emery and Rimoin's Principles and Practice of Medical Genetics and Genomics, 121–147.

Kumar A, Sharma E, Marley A, Samaan MA, Brookes MJ. 2022. Iron deficiency anaemia: Pathophysiology, assessment, practical management. BMJ Open Gastroenterology **9**(1), e000759.

Kumar N, Singh AK. 2016. Role of zinc in male infertility: Review of literature. Indian Journal of Obstetrics and Gynecology Research **3**(2), 167–171.

Liang H, Miao M, Chen J, Chen K, Wu B, Dai Q, Yuan W. 2016. The association between calcium, magnesium, and ratio of calcium/magnesium in seminal plasma and sperm quality. Biological Trace Element Research **174**, 1–7.

Liu P, Yuan G, Zhou Q, Liu Y, He X, Zhang H, Chen J. 2020. The association between metal exposure and semen quality in Chinese males: The mediating effect of androgens. Environmental Pollution **264**, 113975.

Machen GL, Sandlow JI. 2020. Causes of male infertility. Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants, 3–14.

Maciejewski R, Radzikowska-Büchner E, Flieger W, Kulczycka K, Baj J, Forma A, Flieger J. 2022. An overview of essential microelements and common metallic nanoparticles and their effects on male fertility. International Journal of Environmental Research and Public Health **19**(17), 11066.

Mahdi BM. 2021. Semen analysis and insight into male infertility. Open Access Macedonian Journal of Medical Sciences **9**(A), 252–256.

Milachich T, Dyulgerova-Nikolova D. 2020. The sperm: Parameters and evaluation. Innovations in Assisted Reproduction Technology **3**, (no page given).

Morabbi A, Karimian M. 2024. Trace and essential elements as vital components to improve the performance of the male reproductive system: Implications in cell signaling pathways. Journal of Trace Elements in Medicine and Biology, 127403.

Oehninger S, Kruger TF. 2021. Sperm morphology and its disorders in the context of infertility. F&S Reviews **2**(1), 75–92.

Onnarakatt D, Vignesh Lakshmanan K, Priya DAM, Poongothai M. Impact of seminal components on seminal quality. (Incomplete citation details – please provide journal, volume, pages, and year).

Osadchuk L, Kleshchev M, Danilenko A, Osadchuk A. 2021. Impact of seminal and serum zinc on semen quality and hormonal status: A population-based cohort study of Russian young men. Journal of Trace Elements in Medicine and Biology **68**, 126855.

Panner Selvam MK, Ambar RF, Agarwal A, Henkel R. 2021. Etiologies of sperm DNA damage and its impact on male infertility. Andrologia **53**(1), e13706.

Płaczkowska S, Rodak K, Kmieciak A, Gilowska I, Kratz EM. 2024. Exploring correlations: Human seminal plasma and blood serum biochemistry in relation to semen quality. PLOS ONE **19**(6), e0305861.

Plante L, Shepherd LD, King WA, Plante C. 1994. Cleavage and ^{3}H-uridine incorporation in bovine embryos of high in vitro developmental potential. Molecular Reproduction and Development **39**(4), 375–383.

Prasad AS. 2014. Impact of the discovery of human zinc deficiency on health. Journal of Trace Elements in Medicine and Biology **28**(4), 357–363.

Prashanth L, Kattapagari KK, Chitturi RT, Baddam VRR, Prasad LK. 2015. A review on role of essential trace elements in health and disease. Journal of Dr. YSR University of Health Sciences **4**(2), 75–85.

Prien SD, Lox CD, Messer RH, DeLeon FD. 1990. Seminal concentrations of total and ionized calcium from men with normal and decreased motility. Fertility and Sterility **54**(1), 171–172.

Rajalakshmi M, Sharma RS, David GFX, Kapur MM. 1989. Seminal fructose in normal and infertile men. Contraception **39**(3), 299–306.

Rizkalla SW. 2010. Health implications of fructose consumption: A review of recent data. Nutrition & Metabolism **7**, 1–17.

Saleh BOM, Hussain NK, Majid AY, Thabet B, Fadhil KA. 2008. Status of zinc and copper concentrations in seminal plasma of male infertility and their correlation with various sperm parameters. Iraq Postgraduate Medical Journal **7**, 76–80.

Sengupta P, Durairajanayagam D, Agarwal A. 2020. Fuel/energy sources of spermatozoa. Male Infertility: Contemporary Clinical Approaches, Andrology, ART and Antioxidants, 323–335.

Shquirat WD, Daghistani HIA, Hamad AWR, Dayem MA, Swaifi MA. 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), 1–10.

Skalnaya MG, Skalny AV. 2018. Essential trace elements in human health: A physician's view. Tomsk: Publishing House of Tomsk State University **224**, 1–222.

Skoracka K, Eder P, Łykowska-Szuber L, Dobrowolska A, Krela-Kaźmierczak I. 2020. Diet and nutritional factors in male (in)fertility—underestimated factors. Journal of Clinical Medicine **9**(5), 1400.

Skoracka K, Eder P, Łykowska-Szuber L, Dobrowolska A, Krela-Kaźmierczak I. 2020. Diet and nutritional factors in male (in)fertility—underestimated factors. Journal of Clinical Medicine **9**(5), 1400.

Soliman A, De Sanctis V, Elalaily R. 2014. Nutrition and pubertal development. Indian Journal of Endocrinology and Metabolism **18**(Suppl 1), S39–S47.

Soliman AT, De Sanctis V, Yassin M, Soliman N. 2017. Iron deficiency anemia and glucose metabolism. Acta Bio Medica: Atenei Parmensis **88**(1), 112.

Soliman AT, Yasin M, El-Awwa A, Abdelrahman MO, De Sanctis V. 2013. Does blood transfusion affect pituitary gonadal axis and sperm parameters in young males with sickle cell disease?. Indian Journal of Endocrinology and Metabolism **17**(6), 962–968.

Sørensen MB, Bergdahl IA, Hjøllund NHI, Bonde JPE, Stoltenberg M, Ernst E. 1999. Zinc, magnesium and calcium in human seminal fluid: Relations to other semen parameters and fertility. Molecular Human Reproduction **5**(4), 331–337.

Talluri TR, Mal G, Ravi SK. 2017. Biochemical components of seminal plasma and their correlation to the fresh seminal characteristics in Marwari stallions and Poitou jacks. Veterinary World **10**(2), 214.

Taravati A, Tohidi F. 2016. Association between seminal plasma zinc level and asthenozoospermia: A meta-analysis study. Andrologia **48**(6), 646–653.

Tielemans E, Heederik D, Burdorf A, Loomis D, Habbema DJ. 1997. Intraindividual variability and redundancy of semen parameters. Epidemiology **8**(1), 99–103.

Toragall MM, Satapathy SK, Kadadevaru GG, Hiremath MB. 2019. Evaluation of seminal fructose and citric acid levels in men with fertility problem. Journal of Human Reproductive Sciences **12**(3), 199–203.

Trang NT, Huyen VT, Linh NT, Sang TT. 2018. Seminal fructose concentration in man infertility and the fructose test's meaning in diagnosis reason of azoospermia man. Biomed Journal of Scientific & Technical Research **8**(1), 6270–6274.

Trang NT, Sang TT, Hoang N, Khanh NTG, Duc TT. 2018. Assessment of the level of seminal zinc and fructose concentration in seminal plasma of Vietnamese infertile men. International Journal of Research Science and Management **5**(7), 71–82.

Tsao CW, Liao YR, Chang TC, Liew YF, Liu CY. 2022. Effects of iron supplementation on testicular function and spermatogenesis of iron-deficient rats. Nutrients **14**(10), 2063.

Tvrda E, Peer R, Sikka SC, Agarwal A. 2015. Iron and copper in male reproduction: A double-edged sword. Journal of Assisted Reproduction and Genetics **32**, 3–16.

Vanderhout SM, Panah MR, Garcia-Bailo B, Grace-Farfaglia P, Samsel K, Dockray J, El-Sohemy A. 2021. Nutrition, genetic variation and male fertility. Translational Andrology and Urology **10**(3), 1410.

Wang C, Mbizvo M, Festin MP, Björndahl L, Toskin I. 2022. Evolution of the WHO "Semen" processing manual from the first (1980) to the sixth edition (2021). Fertility and Sterility **117**(2), 237–245.

Wise T, Lunstra DD, Rohrer GA, Ford JJ. 2003. Relationships of testicular iron and ferritin concentrations with testicular weight and sperm production in boars. Journal of Animal Science **81**(2), 503–511.

Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM, Copius-Peereboom JH, Steegers-Theunissen RP. 2001. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. Reproductive Toxicology **15**(2), 131–136.

Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM, Copius-Peereboom JH, Steegers-Theunissen RP. 2001. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. Reproductive Toxicology **15**(2), 131–136.