

## Correlation of Antisperm Antibodies with Trace Elements in Seminal Fluid of Immunologic Infertile Men

Abdul Kareem M. Jewad<sup>1</sup>, Ehab Rasmi Alkhafji<sup>2</sup>, Naael H Ali<sup>3\*</sup>

1. Department of Medical Laboratory Technology, College of Health and Medical Technology, Southern Technical University, Basrah, Iraq.

2. Health Department of Dhi-Qar

3. College of Medicine, University of Basrah.

\*Corresponding: Prof Dr. Naael H Ali, [nhali66@yahoo.com](mailto:nhali66@yahoo.com) Tel: 009641308054

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### ABSTRACT:

Immunological cause may contribute to 5-15% of the male infertility factors, including cryptorchidism, primary testicular failure, testicular trauma, epididymitis, varicocele, idiopathic infertility, and infections. Therefore, ASA can be found in primary or secondary infertile men. The aim is to study the correlation of ASA with trace elements in primary and secondary infertile men. The study was carried out in the Department of Medical Laboratory Technology in Southern Technical University. Seminal fluids were collected from each of them, seminogram test (semen analysis) was performed for each participant, and all semen samples were collected following (3-5) days of abstinence. After liquefaction, sperm concentration, total sperm count, morphology, motility grades were determined using World Health Organization (WHO) standard procedures. The mean values of were significantly increased ASA in infertile men as compared to fertile control group ( $p < 0.0001$ ), sperm concentration significant decrease in infertile men as compared to control group ( $p < 0.05$ ) and significant decrease in all sperm motility, total progressive sperm (A Rapid progressive motility, B Slow or sluggish progressive motility and C Non-progressive motility and D Immotility) and normal sperm morphology in infertile men as compared to fertile control group ( $P < 0.05$ ). Decrease in infertile compared to fertile men was statistically significant ( $p < 0.05$ ), while Cu highly increase in infertile men group compared to fertile group highly significant ( $p < 0.001$ ), decrease in Zn infertile men compared to fertile men highly significant ( $p < 0.001$ ). In addition, decrease in Ni in infertile men as compared to fertile group was highly significant ( $p < 0.001$ ).

### Keywords:-

Male infertility, antisperm antibody, immunological infertility, Furnace atomic absorption, selenium, zinc, copper, nickel.

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#### **INTRODUCTION**

Immunologic infertility is probably if more than 50 percent of sperm are bound to IgG or IgA antibodies. It can be assumed if more than that 10% of spermatozoa are antibody bound. These immunoglobulins can be found in men and women and serum, semen and cervical mucus. The incidence of sperm autoimmunity in infertile couples is 9-36 percent, compared to 0.9-4 percent in the fertile population (1). The incidence sperm autoimmunity in infertile couples is 9-36% in contrast to 0.9-4% in the fertile population (1). The incidence of detection of sperm antibodies in the fertile male is 8-21%. The immunological cause may contribute to 5-15% of the male infertility factors (2), including cryptorchidism, primary testicular failure, testicular trauma, epididymitis, varicocele, idiopathic infertility, and infections (3,4). Therefore, ASA can be found in primary or secondary infertile men. Many trace elements are present in human semen, for instance, selenium (Se), copper (Cu), and zinc Zn). They play an important role in scavenging reactive oxygen species as an integral part of Cu/Zn superoxide dismutase and selenium-glutathione peroxidase (5,6) Selenium is essential for normal spermatogenesis of mammals and plays a critical role mainly mediated by two selenoproteins, namely phospholipid hydroperoxide glutathione peroxidase and selenoprotein. (7), and sperm defects during spermiogenesis and maturation in the epididymis (8,9). A matter, which may lead to reduce testis size, and during prolonged deficiency, produces atrophy of the seminiferous epithelium (8). Zinc is essential in the maintenance of germ cells and the progression of spermatogenesis (10). Which is required for the action of more than 200 metalloenzymes and plays an important role in the polymeric organization of macromolecules like DNA and RNA, protein synthesis, and cell division (11)? Mankad and others (12) resulted that zinc, copper, and selenium levels in seminal plasma are associated with sperm quality. Nickel (Ni) enhances reproductive performance and involved in the physiological characteristics of spermatozoa (13). On the other hand, antisperm antibodies (ASA) as a biomarker for immunological infertility have been associated with a decrease in seminal parameters (14). However, there is little information about Se, Cu, Zn and Ni concentration in semen in patients with immunological infertility and their correlation. Its association with seminal parameters (15). This study aimed to determine the concentrations of Se, Cu, Zn and Ni in the semen of patients with immunological infertility indicated by ASA and their correlation.

#### **MATERIALS AND METHODS**

**Study population** This case-control study was carried out in the Basrah Infertility Center and Department of Medical Laboratory Technology in Southern Technical University, Basrah (southern of Iraq), throughout the period from October 2018 to April 2019. The included infertile men were (66) their age ranged from (27-31) years. They suffered from unexplained infertility when they failed to conceive over 12 months of marriage. They being found with not cause for their infertility after workup including: Semen analysis ranged from oligo-normozoospermic, had no overt signs of acute urethritis or, prostatitis, no previous urogenital operations nor any varicocele grades, not infected with mumps previously. Normal hormonal study. Not received corticosteroids or clomiphene citrate, neither any antibiotics before collection samples. The men with a known cause of infertility or have azoospermia were excluded from the study. Exclusion criteria included (oligoasthenoazoospermia or azoospermia, a history of epididymo-orchitis, prostatitis, genital surgery; genital disease or varicocele, presence of any endocrinopathy; of taking alcohol patients were also excluded from analysis known to be associated with decreased fertility; hepatobiliary disease; significant renal insufficiency ,diabetes mellitus and patients taking drugs like vitamin E or vitamin C and glutathione as supplementation within the past three months, smokers and those who refused participation were also excluded from the study as well. Fertile men should have at least recently one child without assisted reproductive their age ranged (17-40) year was considered as a control group. **Samples collection:** Semen samples were collected by masturbation into a sterile, wide-mouthed container, after at least 72 hours (3- 5days) of sexual abstinence. Samples were allowed to liquefy at temperature (37°C) for at least 30 minutes. After

liquefaction, by using light microscopy to determine sperm concentration, sperm motility, sperm grade activity (progressive sperm motility (A), non-progressive sperm motility (B), and immotile sperm (C), total progressive sperm, normal sperm morphology, sperm agglutination, and round cells count, (leukocytospermia) according to World Health Organization (WHO) (15) guidelines. After semen analysis, seminal plasma was separated from spermatozoa by centrifugation at 3500 rotations per minute (rpm) for 15 minutes and stored at (-20 °C) until analysis. Determination of seminal plasma selenium, copper and zinc concentration: Total seminal plasma selenium, copper, zinc and nickel concentration was measured by furnace atomic absorption spectrometry (ASC-7000 Shimadzu – Japan) according to (16). Samples were digested by adding nitric acid and diluted in high purity water (1:2). Wavelength was 324.8 nm. Calibration selenium, copper and zinc were delineated using suitable standard concentrations (10, 50 and 100 mg/L) by diluting standard CuCl<sub>2</sub>, H<sub>2</sub>O solution (Merck, Darmstadt) (16).

**Detection of Seminal Antisperm antibodies:**

ELISA kit (Demeditec Diagnostics GmbH, Germany, ELA 1021 ) was used to detect Antisperm Antibodies ( ASA) in seminal plasma of all participants, by full automated BioTek ELISA (Germany).

**Statistical Analysis**

The data were analyzed using SPSS software (Version 23.0), and the values were expressed as the mean values ± SD. P- values <0.05, 0.01 were considered to be statistically significant (Standard deviation), Range: P-value: N.S (P > 0.05), S (P < 0.05), HS (P < 0.01) indicate the level of significance.

**RESULTS**

**Table1: Seminal fluid parameters between both patients’ infertile and fertile men.**

Parameters	Primary Infertile men (N=33) (Mean ± SD ,range)	Fertile men (N=30) (Mean ± SD, range)	P Value	Secondary Infertile men (N=33) (Mean ± SD ,range)	P Value
Seminal ASA (U/ml)	67.16±16.33 42.03-98.43	42.52±13.67 49.02-93.87	0.0001	64.23±14.89 15.52-60.92	0.0001
Sperm concentration (10 <sup>6</sup> /mL)	117.83±60.03 0-289	50.64±54.25 30-250	0.0001	76.52±56.79 6-230	0.005
Grade A % Rapid progressive motility	28.40±2.81 0-35	15.91±9.92 25-34	0.0001	14.09±11.63 0-40	0.0001
Grade B % Slow or sluggish progressive motility	59.00±6.20 0-50	22.41±12.59 50-70	0.0001	31.70±14.74 0-60	0.0001
Grade C % Non-progressive	6.67±3.24 0-52	27.10±10.28 2-12	0.0001	16.24±13.15 5-50	0.0001

<b>motility</b>					
<b>Grade D %</b>	5.93±3.31	31.86±18.76	0.0001	37.97±19.04	0.0001
<b>Immotility</b>	0-80	1-12		10-95	
<b>Normal morphology</b>	71.47±8.43	32.73±19.68	0.0001	47.12±15.10	0.0001
	0-65	60-88		5-75	

Table 2: concentration of parameters trace elements between both primary and secondary infertile men and fertile men:

Parameters	Primary Infertile men (N=33) (Mean ± SD ,range)	Fertile men (N=30) (Mean± SD ,range)	P Value	Secondary Infertile men (N=33) (Mean ± SD, range)	P Value
Se(ppb)	71.41±5.34 70-99	94.04±3.05 90-98	0.0001	89.34±8.74 61-79	0.004
Cu(ppb)	37.28±10.49 10-29	10.76±3.22 5-15	0.0001	20.76±5.43 17-57	0.0001
Zn(ug/ml)	2.660±1.11 40-4.73	3.035±0.61 2.01-3.83	0.097	2.291±0.85 0.36-3.85	0.001
Ni(ppb)	18.45±4.05 10-27	25.55±4.01 15-30	0.0001	20.26±4.96 10-29	0.0001
Seminal ASA (U/ml)	67.16±16.33 42.03-98.34	42.52±13.67 49.02-93.87	0.0001	64.23±14.89 15.52-60.92	0.0001

Table3: Pearson correlation (R) between antisperm antibody and seminal fluid parameters.

ASA	Sperm Concentration	A	B	C	D
R	-0.220-	- 0.297	- 0.445	0.343	0.364
P value	0.031	0.003	0.0001	0.001	0.0001

Table4: Pearson correlation (R) between antisperm antibody and trace elements:

ASA	Se	Cu	Zn	Ni
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<b>R</b>	<b>-0.361-</b>	<b>0.406</b>	<b>-0.120-</b>	<b>0.348-</b>
<b>P value</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.243</b>	<b>0.001</b>

Table 1 shows seminal fluid parameters between patients infertile and fertile men of studied groups, In the current study antisperm antibody ASA was present in the seminal plasma of the unexplained infertility was highly significant ( $p < 0.0001$ ) is compared with the infertile (control). The same significant difference with secondary significant ( $p < 0.0001$ ) is compared with the fertility (control). Results of semen analysis for the primary infertile men and the fertile revealed highly significant difference ( $P < 0.001$ ) in sperm concentration while in secondary infertile as compared with fertile men significant difference was resulted ( $P < 0.05$ ) in sperm concentration and in both primary and secondary infertile group compared with the fertile group significant difference ( $P < 0.05$ ) in all sperm motility, total progressive sperm (A Rapid progressive motility ,B Slow or sluggish progressive motility and C Non-progressive motility and D Immotility) and normal sperm morphology shown in table (1). This high rate of ASAs indicated that one cause of infertility was immunological. In the current study positive ASA of seminal plasma samples showed an apparent decrease in the motility; these result were observed in table(1), the immune response created by ASAs was associated with obvious changes in the standard parameters and alterations of the quality of the semen, which agrees with a study by ((19,20). This means that ASAs develop when the patient's immune system identifies the sperm cells as a result of a disruption in its environment because sperm-specific antigens are not present during the development of immunological tolerance

Our results of seminal fluid parameters between primary infertile and fertile men were an agreement with the result of (21, 22, 20).

In the table (2) there is a decrease in seminal selenium concentration in both primary and secondary groups compared to fertile group significant ( $p < 0.05$ ). and , the seminal copper concentration showed highly increase in primary and also increase in secondary infertile men group compared to fertile group highly significant ( $p < 0.001$ ),and also there is a decrease in seminal zinc concentration in primary groups compared to fertile group but non-significant ( $p > 0.05$ ) while decreasing seminal zinc concentration in secondary groups compared to fertile group highly significant ( $p < 0.001$ ). and also there is a decrease in seminal nickel concentration in both primary groups and secondary compared to fertile group highly significant ( $p < 0.001$ ) and an increase in seminal antisperm antibody(ASA) concentration in both primary and secondary groups compared to fertile group highly significant ( $p < 0.001$ ).

The results of the present study show a significantly low concentration of selenium in both primary and secondary when compared with that of control fertile group. The study conducted by (23)(24); (25),(26); also agree with our findings that low-level seminal plasma selenium concentration was observed in infertile groups. (27), (28), (29) observed a significant increase of selenium concentration in azoospermic, when compared with oligozoospermic subjects and normozoospermic contrast to our findings. Our results demonstrated that there was a positive significant correlation between selenium and semen quality. Our findings are not agreed with those of other studies conducted by(30),(31)but agreed with(26).

Seminal zinc levels are higher in men with control fertile compared to men with patients infertility and seminal zinc levels are highly correlated with total sperm count (34;33;32). Zinc supplementation increases total sperm count and decreases anti-spermatozoa antibodies in men with asthenospermia (35). Likewise, in a double-blind, placebo-controlled trial in men with OAT, combination folate and zinc supplementation were found to increase sperm concentration (36). But agree (37).

Zinc levels in human seminal plasma have been noted to be remarkably high in comparison with other body fluids (38). However, the exact mechanism in the function of zinc in the reproductive system is still unclear. Lower zinc concentrations in semen have been reported in infertile men (39). We found that zinc levels were low in all biological fluids in infertile men. But agree (37).

High copper concentration was observed in infertile and low concentration was seen in control fertile men. Similar results by (40)(41) show significant correlations between seminal plasma copper concentration and sperm concentration, pH, vitality, motility and normal morphology. (42) showed that copper levels in seminal plasma infertile men participants were significantly higher than those in control fertile participants. (43) found significant correlations between poor semen quality and copper concentration. (44) demonstrated that higher concentrations of copper had significant adverse effects on sperm motility which is supportive of our results. (45)

Nickel (Ni) concentration was significantly low in infertile and fertile men. An experimental studies proved beneficial of Ni for reproductive performance; deficiency of Ni diminished physiological characteristics of spermatozoa, which most likely could result in impaired reproductive ability (46). Ni likely affects sperm motility by changing CNG cation channel function (47), CNG cation channel involved in spermatozoa capacitation through providing the influx of  $Ca^{2+}$  to the cytoplasm during capacitation. Deficiency in Ni concentration leads to impaired CNG channel and insufficiency incapacitation (48). Conducted experimental studies were proved beneficial of Ni for reproductive performance; deficiency of Ni diminished physiological characteristics of spermatozoa, which most likely could result in impaired reproductive ability (46).

Ni deficiency significantly decreases spermatozoa production rate of the testes, spermatozoa density in the epididymis, epididymis transit time, and spermatozoa motility (49, 46). Ni likely affects sperm motility by changing CNG cation channel function (47), CNG cation channel involved in spermatozoa capacitation through providing the influx of  $Ca^{2+}$  to the cytoplasm during capacitation. Deficiency in Ni concentration leads to impaired CNG channel and insufficiency incapacitation (48). Spermatozoa acrosome reaction induced by oligosaccharide ligands on the zona surface and sperm surface sugar receptors (mannose-ligand receptor); surface receptors are involved in the prevention of acrosome reaction (50). Ni is involved in the activation of mannose-ligand receptor binding (50), Decreased Ni concentration may inactivate mannose-ligand receptor and prevent acrosome reaction, this may explain control fertile group is infertile even they have normal sperm parameters.

Nickel deficiency can also harm spermatogenesis and semen quality. In the experiment with rats supplemented by dietary (46) found a significant decrease in the density of epididymides, the epididymis transit time of spermatozoa, testes sperm production rate, and weight of seminal vesicles and prostate. Agree (51).

In table.3 Seminal ASA levels correlated negative significantly ( $r=-0.220$ ,  $0.031$ -;  $p<0.05$ ) with normal concentration count, Seminal ASA levels correlated negative significantly ( $r=-0.297$ -,  $0.003$ -;  $p<0.05$ ) grade A progressive, , Seminal ASA levels correlated negative highly significantly ( $r=-0.445$ -,  $0.001$ -;  $p<0.05$ ) grade B progressive, Seminal ASA levels correlated positive highly significantly ( $r=0.343$ ,  $0.001$ -;  $p<0.05$ ) grade C, . In addition, seminal plasma ASA levels were correlated highly significantly and positively ( $r=0.364$ ;  $p<0.001$ ) with grade D.

In table 4. Seminal ASA levels correlated negative highly significantly ( $r=-0.361$ -;  $p<0.0001$ ) with seminal selenium concentration, Seminal ASA levels correlated positive highly significantly ( $r=-0.406$ -;  $p<0.0001$ ) with seminal copper concentration, also Seminal ASA levels correlated negative but not significantly ( $r=-0.120$ --;  $p>0.05$ ) with seminal zinc concentration, Seminal ASA levels correlated negative highly significantly ( $r=0.348$ -;  $p<0.001$ ) with seminal nickel concentration.

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