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Research Article
Effects of Trace Metals Levels and Hyaluronic Acid Degrading Enzymes Activities on Human Sperm Function

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Abstract

Background and Objective: Deficiency of trace elements that are cofactors of fertilizing enzymes can cause sperm dysfunction and male infertility. In the present work, the levels of some trace metals (Cu, Zn and Mn) and the activities of hyaluronic acid degrading enzymes were evaluated in semen from fertile and infertile men. **Materials and Methods:** Two hundred and sixteen semen samples were divided into 5 groups: Group 1 (G1) was healthy controls and another 4 infertile group 2-5 according to WHO criteria. Levels of Cu, Zn and Mn and activities of hyaluronidase (Hase), N-acetyl-β-D-glucosaminidase (NAG) and β-D-glucuronidase (β-Gluc) were estimated in the sperms and seminal plasma of these groups. **Results:** Significant decreases in Cu^{2+} and Zn^{2+} contents were observed in seminal plasma and sperms homogenate of the infertile groups. Moreover, the activities of the 3 hyaluronic acid degrading enzymes in sperms homogenate supernatant of infertile males were highly reduced compared to the control group. The Hase activity in seminal plasma of all groups was completely absent. The activity of β-Gluc in seminal plasma of infertile males was highly elevated than control group. **Conclusion:** The disturbance in levels of trace metals and the hyaluronic acid degrading enzymes activities are associated with human male infertility and may be useful tools in predicting semen quality.

Key words: Seminal plasma, sperms, human fertility, hyaluronic acid degrading enzymes, trace metals

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The semen quality may be affected by many environmental factors, such as, nutritional, occupational exposure or other unknown factors[1]. Trace elements may play an important role in the male reproductive process. The levels of trace elements in the semen have a correlation with the sperm parameters[2]. Many of trace elements act as co-factors for several enzymes. The metal ions copper, zinc, iron and selenium act as co-factors for the antioxidant enzymes such as the Cu, Zn-superoxide dismutase, catalase and glutathione peroxidase, respectively. These enzymes play important protective role for semen. Their presence determines the respective antioxidant enzyme activity[3]. Therefore, trace elements play major roles in male fertility and directly affect sperm quality[4].

Another factor that can potentially cause spermatozoal dysfunction is the inefficient binding to zona pellucida (ZP) for producing acrosomal reaction. The ultimate goal of spermatozoa is the successful fertilization of oocyte resulting in normal conception. The oocyte is enclosed within a glycoprotein envelope called the ZP and surrounded by the cumulus cells which are held together by a hyaluronic acid matrix[5]. Hyaluronic acid not only surrounds the ZP as a part of the cumulus matrix but also present throughout ZP and the perivitelline space of the oocytes[6]. The acrosome cap at the interior of the sperm head include several hydrolytic enzymes such as hyaluronic acid degrading enzymes which assist the sperm in penetrating the accessory layers that surround the oocyte and containing hyaluronic acid[7]. Hyaluronidase (Hase) is considered to be the most important key enzyme that facilitates the sperm-egg interaction through its participation in the degradation of hyaluronic acid matrix. This degradation results in a tetrasaccharide[8] that can be further degraded by N-acetyl-β-D-glucosaminidase (NAG) and β-D-glucuronidase (β-Gluc). From all the previous studies it is clear the biological role of trace elements and hyaluronic acid degrading enzymes in sperm function.

In the present study, the levels of trace elements (Cu, Zn and Mn) as well as the activities of hyaluronic acid degrading enzymes in semen of infertile males had been examined.

MATERIALS AND METHODS

The project had been performed in collaboration between the Department of chemistry, Faculty of Science, Damietta University, Faculty of Medicine, Mansoura University, Egypt and Department of Biochemistry, Faculty of Science, University of Tabuk, Saudi Arabia. The ethical committee of the Faculty of Science, Damietta University, has approved this project.

Samples collection: Semen samples were collected from a private hospital before 2015. They were collected after 3 days of sexual abstinence and the parameters [morphology and motility] grades were analyzed by a sperm analyzer (CASA, Cell Soft 3000, Cryo Resources Co., U.S.A.) as described in a previous study by Habib *et al.*[9]. The practical part was carried out in biochemistry laboratory of Faculty of Science, Damietta University, Damietta, Egypt.

Samples grouping: Two hundred and sixteen human semen samples were divided into 5 groups based on WHO criteria[10]. Group 1 (G1): 70 normal semen samples as control and 4 groups from infertile male. Group 2 (G2): 48 asthenozoospermic (A) samples having defects only in their sperm motility (<50%). Group 3 (G3): 18 oligo-asthenozoospermic (OA) samples having defects in both sperm density (<20 million mL^{-1}) and motility (<50%). Group 4 (G4): 41 oligo-astheno-teratozoospermia (OAT) samples having defects in all semen parameters including sperm density (<20 million mL^{-1}), motility (<50%) and normal morphology (<30%). Group 5 (G5): 39 azoospermic (Azoo) samples having no spermatozoa at all in their semen.

Semen preparation: The semen samples were prepared for analysis as described in previous study[9].

Biochemical analysis: Hase activity was assayed by the standard method[11]. The NAG activity was assayed by a modified method[12] and β-Gluc activity was assayed by the standard method[13]. Trace elements were determined by atomic absorption spectrophotometery[14]. Protein electrophoresis was carried out using polyacrylamide gel electrophoresis[15].

Statistical analysis: The statistical analysis of the results was carried out using Instate software computer program, version 2.03 (Graph pad, USA), Origin software computer program, Inc. version 6.0 (Northampton, Ma 01060 USA) and Gel Pro Analyzer program (Media Cybernetices, Georgia, USA). All parameters were expressed as Mean±Standard deviation (SD).

RESULTS

Trace metals in seminal plasma: Zinc and copper contents in seminal plasma of all infertile groups (G2-5) were highly significantly decreased compared to the controls, while, manganese content in group 4 and 5 only was highly significantly decreased compared to the control (Table 1).

Trace metals in spermatozoa homogenate supernatant: In Table 2, the levels of Zn and Cu in sperm homogenate supernatant of G2 and 3 were significantly decreased compared to the corresponding controls. However, the concentrations of the 3 trace metals ions (Zn, Cu and Mn) in sperm homogenate supernatant of G4 were highly significantly increased compared to the corresponding controls.

Hyaluronic acid degrading enzymes in seminal plasma: The results in Table 3 indicate that, the activity of Hase is completely absent in seminal plasma of all groups and the activity of β-Gluc in G2-5 has no significant change than the control. However, the activity of NAG in seminal plasma of G2-4 was highly increased compared to the control.

Hyaluronic acid degrading enzymes in sperm homogenate supernatant: The activities of the 3 hyaluronic acid degrading enzymes (β-Gluc, Hase and NAG) in sperm homogenate supernatant of infertile groups (G3, 4) were highly significantly decreased compared to the controls (Table 4).

Motility and morphology: In Table 5, the total progressive sperm motility in group 2-4 was highly significantly decreased compared to the control. However, the abnormal sperm morphology of group 2-4 was highly significantly increased compared to the control.

Electrophoresis and gel pro-analysis of sperms homogenate protein: A comparison between the protein in motile and immotile sperm cells homogenates of normozoospermic (control) and asthenozoospermic patient (G2) are indicated in Fig. 1a-e.

It can be seen that, the protein bands of motile sperms homogenate are sharp and more intense than the immotile sperms homogenate protein bands (Fig. 1a-c).

Table 1: Mean values of Zn, Cu and Mn in seminal plasma of group 1-5

	Protein (μg mg^{-1})		
Groups	Zn	Cu	Mn
G1 (Control)	3.93±0.76	0.09±0.03	0.087±0.026
n	15	17	10
G2 (A)	1.91±0.56**	0.04±0.02**	0.079±0.022
n	11	10	11
G3 (OA)	1.1±0.29**	0.04±0.02**	0.069±0.02
n	5	6	6
G4 (OAT)	2.17±0.56**	0.03±0.01**	0.055±0.015**
n	15	18	15
G5 (Azoo)	2.13±0.61**	0.06±0.02**	0.045±0.017**
n	15	16	10

**Highly significant, n: Number of cases, values are expressed as Mean±SD

Table 2: Mean values of Zn, Cu and Mn in spermatozoa homogenate supernatant of group 1-5

	Protein (μg×10^2 mg^{-1})		
Groups	Zn	Cu	Mn
G1 (Control)	1.28±0.38	0.07±0.02	0.049±0.019
n	18	15	10
G2 (A)	0.72±0.18**	0.03±0.01**	0.07±0.02*
n	10	10	9
G3 (OA)	0.79±0.18**	0.05±0.01*	0.03±0.01*
n	6	5	6
G4 (OAT)	2.29±0.68**	0.16±0.04**	0.16±0.05**
n	17	16	14
G5 (Azoo) n	No sperms	No sperms	No sperms

*Significant, **Highly significant, n: Number of cases, values are expressed as Mean±SD

Table 3: Activities of Hase, NAG and β-Gluc in seminal plasma of group 1-5

	Parameters		
Groups	Hase (mg mL^{-1}/18 h)	NAG (μM 10^{-3} min^{-1} g^{-1}) protein	β-Gluc (μM 10^{-3} min^{-1} g^{-1}) protein
G1 (Control)	-	3.86±1.15	2.07±0.75
n	-	20	21
G2 (A)		4.8±1.41*	2±0.49
n	-	16	11
G3 (OA)		5.23±1.39**	1.78±0.495
n	-	9	7
G4 (OAT)	-	5.75±1.83**	2.11±0.84
n		11	24
G5 (Azoo)		3.49±1.08	2.05±0.54
n		16	11

*Significant, **Highly significant, n: Number of cases, values are expressed as Mean±SD

Table 4: Activities of Hase, NAG and β-Gluc in spermatozoa homogenate supernatant of group 1-5

	Parameters		
Groups	Hase (mg mL^{-1}/18 h)	NAG (μM 10^{-3} min^{-1} g^{-1}) protein	β-Gluc (μM 10^{-3} min^{-1} g^{-1}) protein
G1 (Control)	16.0±5.4	15.12±4.39	0.389±0.14
n	20	25	22
G2 (A)	13.3±4.8	14.11±3.51	0.181±0.066**
n	12	15	16
G3 (OA)	1.1±0.41**	10.85±2.72**	0.073±0.028**
n	6	10	7
G4 (OAT)	0.115±0.04**	4.05±1.67**	0.02±0.008**
n	16	10	19
G5 (Azoo)	No sperms	No sperms	No sperms
n			

**Highly significant, n: Number of cases, values are expressed as Mean±SD

Table 5: Total progressive sperm motility and abnormal sperm morphology in semen of group 1-5

Groups	Abnormal sperm morphology (%)	Total progressive sperm motility (%)
G1 (Control)	40.1±7.4	62.5±7.1
n	70	70
G2 (A)	47.8±8.8**	27.4±11.5**
n	48	48
G3 (OA)	55.8±7.7**	24.6±11.7**
n	18	18
G4 (OAT)	80.9±9.7**	9.7±10.7**
n	41	41
G5 (Azoo)	No sperms	No sperms
n		

**Highly significant, n: Number of cases, values are expressed as Mean±SD

There is a clear similarity between the Gel-pro-analysis of immotile sperms homogenates of the control group and immotile sperms homogenates of an infertile group (Fig. 1d). However, a clear difference between the gel pro analysis of motile sperms homogenate of the control group and motile sperms homogenate of an infertile group was observed in Fig. 1e.

In Fig. 2, there are clear differences between the Gel-pro-analysis of the control group and the abnormal group (3 and 4) with a little difference between control and group 2 (Asthenozoospermia).

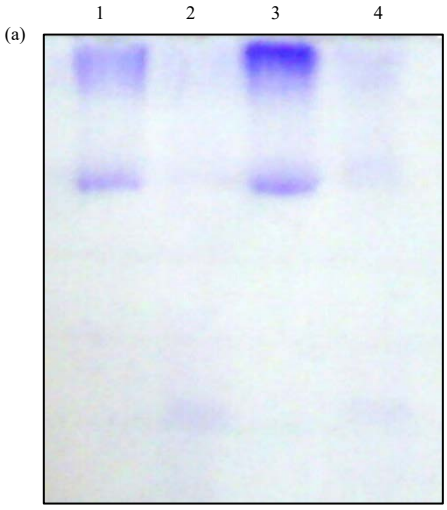
DISCUSSION

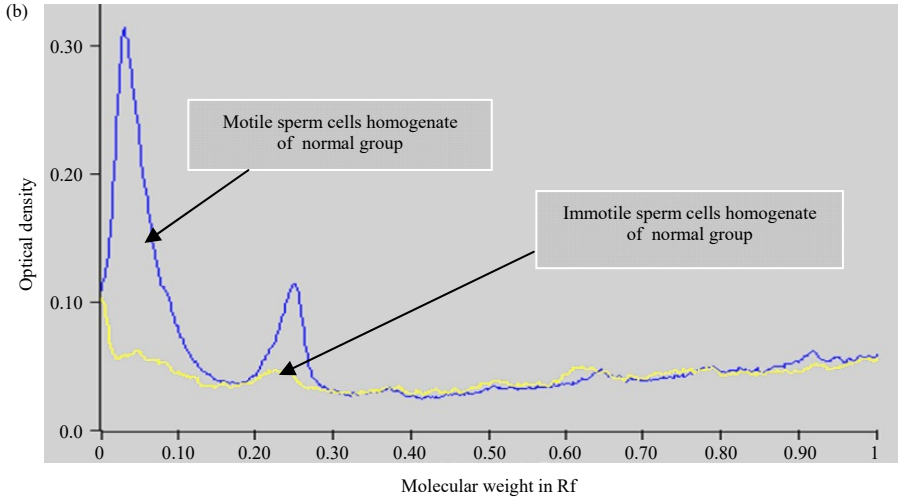
The WHO reported that, more than 30% of humans are deficient in zinc[16]. The most common cause is the insufficient intake due to low Zn content in the diet. Also, during muscle catabolism Zn loses in urine is increased[16,17]. It has been reported that the sperm density or motility are not correlated with Zn concentration in seminal fluid[4]. In contrast, others showed that the decreased in Zn concentration in semen would lead to reduce in numbers and activity of spermatozoa[18,19]. Cu is essential for physiological functions but Cu overload causes cytotoxicity in living organisms including human[20].

In the present results, the levels of Zn, Cu and Mn were decreased in group 2 and 3 and highly increased in G4. In accordance with these results, a recent study showed a significant decrease in seminal plasma zinc content in patients compared to the healthy group[16]. On the other hand, the increased in levels of Zn, Cu and Mn in sperm homogenate of G4 is the cause of high abnormality because certain levels of these trace metals are essential for good sperm quality but below or above these levels would lead to low sperm quality and infertility[21]. In addition, these results are in agreement with the previous studies that showed a correlation between sperm density; motility and viability with zinc concentration in seminal plasma[3,22,23]. The current results are also in accordance with a recent study reported that the concentration of Fe, Cu and Mg are decreased in males with sperm motility problems[4].

The obtained data showed the absence of Hase activity in the seminal plasma of both normal and abnormal cases. This result is expected as the seminal Hase is entirely originated from spermatozoa and specifically localized to the acrosome[24]. Results also showed a reduction in Hase activities in sperm homogenate of infertile groups compared to the control, suggesting that Hase activity is directly associated with the fertility in males.

The NAG is an active glycosidic enzyme found in sperm and its surrounding fluid[25]. Its action is of significance in the reproductive processes[26]. It is considered to be the mediator that induces acrosome reaction in human spermatozoa[27]. In the current study, the activity of NAG in seminal plasma of infertile men (G2-4) was elevated in response to oxidative





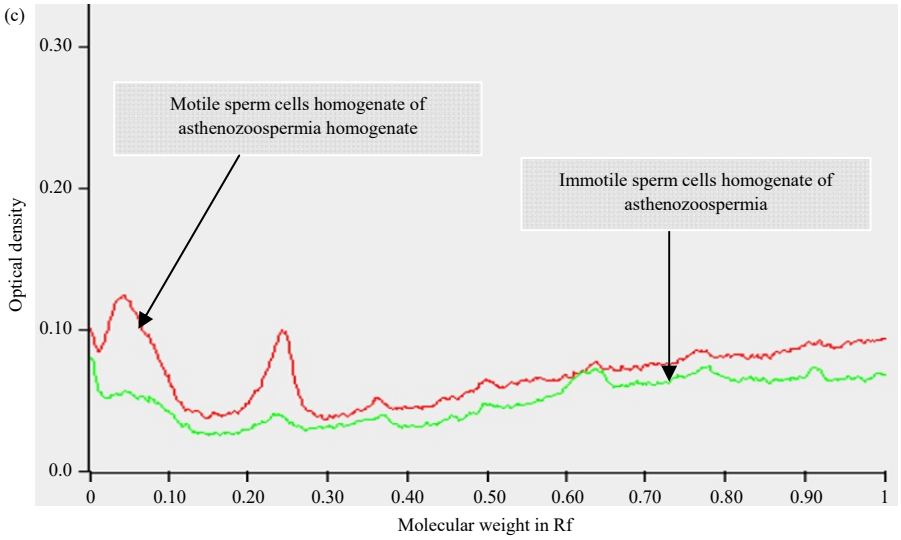


Fig. 1(a-e): Continued

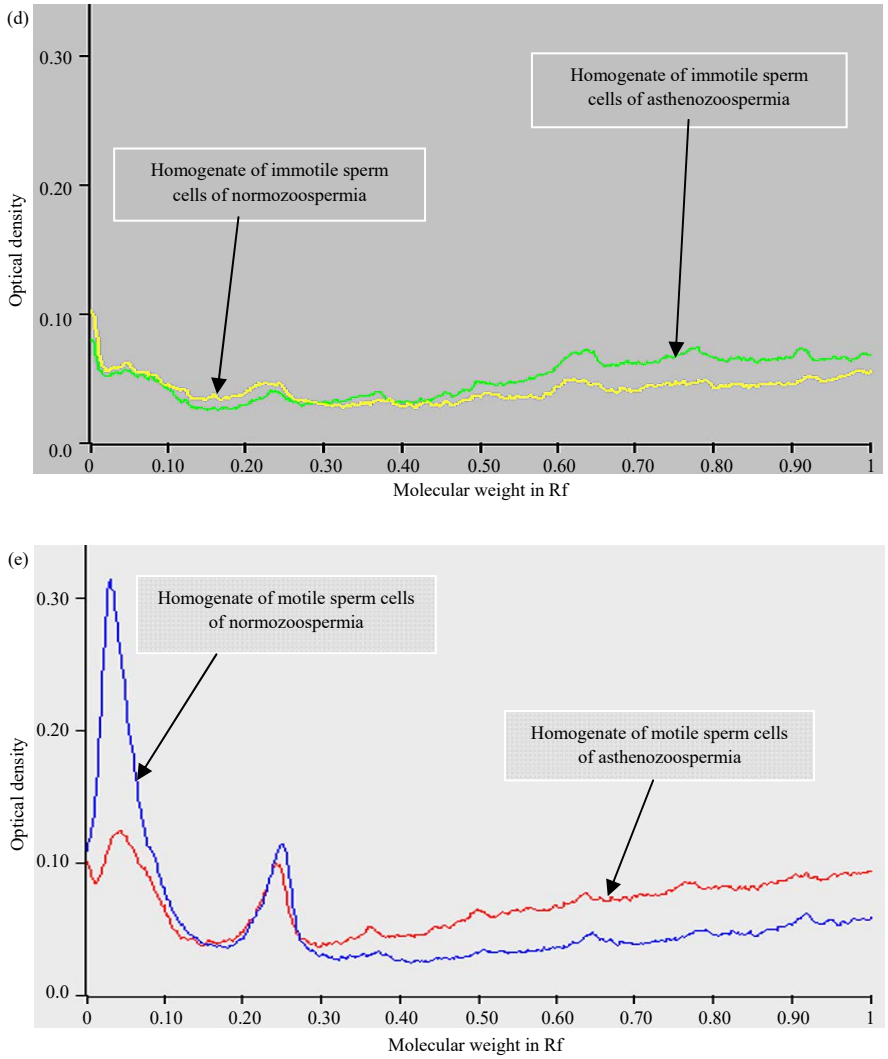


Fig. 1(a-e): (a) PAGE of protein of motile and immotile sperm homogenate from G2 (lane 1 and 2, respectively) and motile and immotile sperm homogenates from G1 (lane 3 and lane 4, respectively), (b) Gel-pro analysis of protein bands in motile (lane 3) and immotile (lane 4) sperm homogenate of G1, (c) Gel-pro analysis of protein in the motile and immotile sperm homogenates of G2, (d) Gel-pro analysis of protein in the immotile sperm homogenate of G1 and immotile sperm homogenate of G2 and (e) Gel-pro analysis of protein in the motile sperm homogenates of G1 and 2

stress, a phenomena that can cause disruption of the sperm plasma membrane causing liberation of the weakly associated enzyme. The presence of NAG activity in seminal plasma of azoospermic men is also expected as NAG is not only secreted by the spermatozoa but also secreted by the epididymis and prostate[28].

The increase in activity of β-Gluc in sperm homogenate than its activity in seminal plasma and the reduction in the activity of β-Gluc in sperms of infertile groups than the control indicated that sperm β-Gluc enzyme is strongly associated with sperm fertilizing capacity[28].

The present results showed no significant differences in β-Gluc activity in seminal plasma of the different groups and these results might be due to the strong binding between β-Gluc and the head of the sperm. The results showed that the activity of NAG is about 38 times higher than activity of β-Gluc in the sperm homogenate of the control group, in accordance with a previous study by Brandelli *et al.*[29].

The seminal quality is affected mainly by the disturbance in protein content of semen[30]. The results of the current study showed that the Gel pro-analysis of proteins in sperms homogenates of control was different from that of the infertile

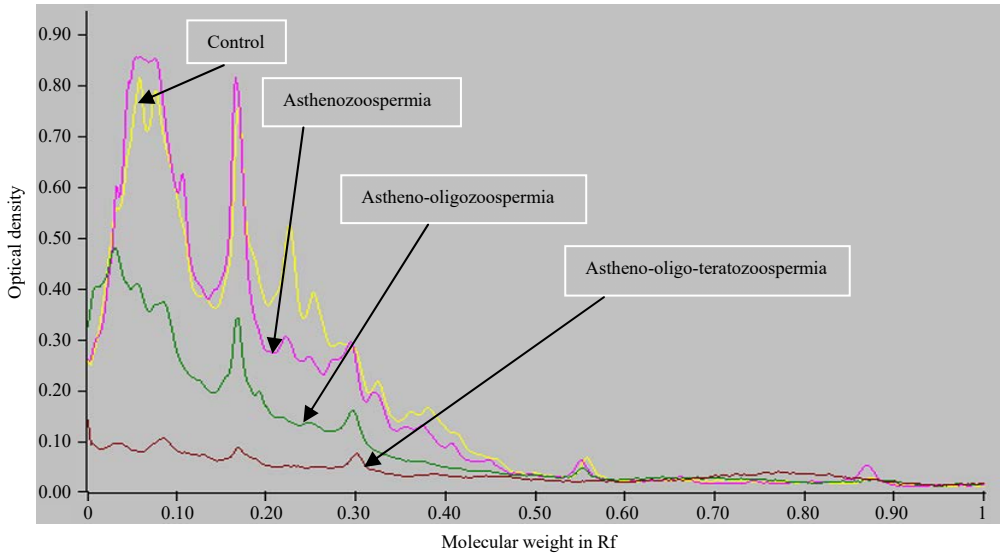


Fig. 2: Gel-pro analysis of sperm cells homogenate proteins of normozoospermic donor (control) and other abnormal groups (asthenozoospermic, astheno-oligozoospermic and astheno-oligo-teratozoospermia)

groups (Fig. 1a-e). Also, there are clear differences between protein peaks of motile sperms in normal and motile sperms in abnormal cases (Fig. 1b, c, e). However, the Gel pro-analysis of protein in sperm homogenates of immotile sperms in control was similar to those of infertile groups (Fig. 1d). In accordance with these results, a recent study by Vickram *et al.*[30] stated that the seminal protein contents have a high effect on the sperm morphology and physiology and therefore its fertility. This effect is very clear in Fig. 2 and Table 5, whereas, the disturbance in semen protein content and sperm morphology increased from G2-5.

CONCLUSION

The human male fertility is highly disrupted by the reduction in trace metals levels especially those play an important physiological role in sperm motility and fertilization activity. In addition, the decrease in activity of hyaluronic acid degrading enzymes is highly correlated with the male infertility. These parameters, in addition to the disturbance in sperm proteins can be used as useful markers for predicting the semen quality.

SIGNIFICANCE STATEMENT

Reduced levels of trace elements (Cu, Zn, Mn) and decreased activities of hyaluronic acid degrading enzymes are highly correlated with male infertility. These parameters, in addition to the disturbance in sperm proteins can be used as useful biomarkers in predicting semen quality.

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