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Toxicology

A novel functional role of nickel in sperm motility and eukaryotic cell growth

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ABSTRACT

Background: Metal ions are essential for numerous life processes. This study aims to investigate the relationship between seminal quality and ion levels in seminal plasma.
Basic procedures: A total of 205 semen samples were collected and seminal plasma ion levels were examined with inductively-coupled plasma-mass spectrometry. The nickel function was demonstrated by in vitro assay and cell growth.
Main findings: The low sperm motility group showed distinctively reduced nickel concentration in seminal plasma compared with the normal sperm motility group. However, arsenic, sulfur, selenium, magnesium and zinc were negatively associated with sperm quality. No significant relationship between other examined cations and semen quality was observed. In vitro assay suggested low concentration of nickel significantly increased sperm total motility and progressive motility. Cell growth assay further confirmed nickel promoted eukaryotic yeast cell growth. Nickel level in seminal plasma may play important functions to determine sperm quality.
Principal conclusions: Our study reveals a strong correlation between S, Mg, Se, Zn, As, Ni and seminal quality as well as discovers a novel functional role of nickel in sperm motility and eukaryotic cell growth. These findings may provide a potential avenue for assessment of sperm quality and treatment of reproduction disorders.

1. Introduction

Metal ions play vital roles in numerous biological pathways, which is reflected by metalloproteins composing approximately one third of the proteome [1–3]. Organisms have evolved delicate mechanisms to control metal acquisition, distribution, utilization and detoxification. Any defect in their homeostasis is attributed to diverse health problems [4–6].

An estimated 6% of adult males are thought to be infertile [7]. The main clinical reasons of male infertility include decreased sperm motility, reduced sperm count and sperm morphological abnormalities [8,9]. More than 85% of infertile males are able to produce sperm [10], but in most of cases, their sperms cannot fertilize an egg. Seminal plasma is microenvironment of sperm, which is a mixture of secretions from the testes, epididymides and accessory sex glands containing inorganic ions, organic acids, sugars, lipids, steroids, amino acids, polyamines, nitrogenous bases and proteins [11]. These inorganic ions include many metal ions like calcium (Ca), magnesium (Mg), copper (Cu), selenium (Se), and zinc (Zn) in bound and free (ionic) forms. Metal ions have crucial effects on various parameters of semen [12].

Excess accumulation of metal ions is highly toxic. Environmental contamination of toxic metals has become one of the most serious health problems in the world [13,14]. Industrial and agricultural activities have increased metal levels on the earth's crust, which leads to numerous metal ion-associated diseases [15,16]. A report of the world health organization indicated that about 24% of diseases and 23% of human deaths could be ascribed to environmental factors, in which environmental pollutants emerge as the greatest danger to public health [17]. Humans are exposed occupationally and environmentally to heavy metals which accumulate in the male reproductive organs to affect fertility and sperm parameters [18–20]. Environmental metal ions may play a part in seminal plasma microenvironment affecting sperm quality.

The ionomics of semen has led to novel insights into ion

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functionality in male fertility [15,18–20]. Previous human studies on the relationship between exposure to metals and male fertility revealed that sperm quality is different depending on geographical location [21–23]. Moreover, geographical variations in semen quality may be influenced by environmental factors, such as climate, and pollution [24–26]. Most of these studies focused on the role(s) of single or a few ions due to methodological limitation [27]. However, the interactions of ions are extremely complicated under various physiological and pathological conditions [28,29]. It remains unclear how a comprehensive ionomic profile reflects outcomes of diseases. By using inductively coupled plasma mass spectrometry (ICP-MS) as a powerful tool owing to its sensitivity and comprehensive analysis of different metal ions at the same time, we are able to better assess the associations of ions with diseases [30].

Seminal plasma is very important for sperm function, metabolism, survival, and transport in the female genital tract. Abnormal levels of ions in seminal plasma are involved in infertility in humans. In the present study, a total of 205 semen samples were collected from Yangtze River Delta Region, in eastern China, a highly developed area with 75 million people in 99,600 square kilometers exposed to a higher amount of pollutants closer to sources of industrialization [13,14,16,31]. By utilizing an advanced ionomic technology, we comprehensively investigated the relations between cation profiles of seminal plasma and sperm quality and identified new function of nickel for sperm activity and eukaryotic cell growth.

2. Materials and methods

2.1. Study participants

A total of 205 eligible human subjects were randomly collected for this study. Administration of the questionnaire and the physical examination, semen collection, and all analyses were carried out at the Reproductive Center of Shanghai Sixth People's Hospital, Shanghai, China. Men were requested to be abstinent for 3–8 days before providing a semen sample. All participants provided written informed consent at enrollment and the study was approved by the Ethics Committee of Shanghai Sixth People's Hospital. The participants voluntarily provided semen samples. They were age 20–50 and permanent residents of the east China area with no medical treatments that might affect spermatogenesis. Conventional semen parameters for the original raw samples, including appearance, viscosity, liquefaction time, pH value, semen volume, sperm concentration, and motion parameters, were measured according to World Health Organization guidelines [32].

2.2. Semen analysis

The study participants provided their semen sample by masturbation into a sterile plastic specimen container in a specialized semen collection room. After liquefaction of the semen sample in a heating chamber (37 °C) for no more than 60 min, semen quality parameters were analyzed using a computer-aided semen analysis system (CASA, WLJY 9000, Weili New Century Science & Tech Dev, Beijing, China) [33,34]. Total semen volume was measured using a serologic pipet with an accuracy of 0.1 ml. To measure sperm concentration and motility, 10 μl of well-mixed semen sample was placed into a pre-warmed (37 °C) Makler counting chamber, covered with a coverslip, and then immediately examined at magnification of 400× using a computer-aided semen analyser. Ten of the 100 squares in the microscope field were randomly scanned and analyzed. Three conventional semen quality parameters reported here are sperm concentration (million/mL), sperm count (million, semen volume × sperm concentration), and sperm motility (% A + B motile sperm). To minimize the variation in the assessment of semen quality parameters, all of the procedures were performed by the same well-trained laboratory technician.

2.3. Determination of metal ions in seminal plasma

Semen samples were centrifuged at 1000 rpm for 5 min to separate sperm and seminal plasma. The seminal plasma samples were digested in 70% nitric acid at 70 °C for 3 h and then overnight at room temperature. Levels of major physiological ions were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent, Model 7500 cs) coupled to a 96-well plate autosampler (Elemental Scientific Inc), which was operated in collision/reaction mode (H2 3.5 mL/min, He 1.5 mL/min) with 50 ppb Ga as an internal standard as described previously [35].

2.4. In vitro effects of Ni^{2+} on sperm total motility and progressive motility

The abnormal semen samples (total motility < 40%) were centrifuged at 1000 rpm for 5 min to collect low activity sperms and diluted to 20×10^6/ml. For the time-course experiment, a 50-μl diluted sperm sample was incubated for 0.5, 1, 1.5, or 2 h in flasks without (control) or with $NiSO_4$. For the concentration-effect experiment, diluted sperm sample was incubated with saline (control) or 0.5, 1, 4, or 10 μM $NiSO_4$ for 0.5–2 h. Sperm total motility and progressive motility were measured and expressed as a percentage.

2.5. Yeast strains, media, and cell growth assays

A haploid control yeast *S. cerevisiae* strain, BY4741 was purchased from the Open Biosystems. Yeast cells were cultured in YPD media (1% yeast extract, 2% Bacto-peptone, and 2% dextrose). Solid media was prepared with the supplementation of 1.5% agar. Yeast cells were cultured at 30 °C.

For growth assay, cells cultured overnight in YPD media were diluted into fresh media (OD_{600} = 0.2) and re-cultured to the mid-log phase (OD_{600} = 0.8–1.0). After dilution to OD_{600} = 0.5 in sterilized water, ~5 μl of cells were spotted on selection media that was supplemented with indicated concentrations of $NiSO_4$. Growth assays were conducted using cells of OD_{600} = 0.5 and 2 × serial dilutions. Plates were incubated at 30 °C for 2 days before photography. Each assay was repeated at least twice using two different colonies to confirm results.

2.6. Statistical analysis

Descriptive analysis was conducted to determine the mean, distribution of demographics, semen quality parameters, and metal ion concentration in seminal plasma. Mean concentration of each metal ion was calculated based on two separate groups of each semen parameter based on the WHO reference values (33). Data were presented as means ± SD. Student's *t*-test determined statistical difference between the groups. $P < 0.05$ was considered to be significant. Normal distribution and clustering analysis were analyzed based on SPSS 22.0 software. The R project for statistical computing software was used to calculate FDR corrected p-value.

3. Results

3.1. Demographic characteristics of the participants

A total of 205 semen samples were collected from Yangtze River Delta Region, in eastern China. According to the WHO 2010 standard, the samples were divided into two groups, normal motility group (total motility > 40% (55.6 ± 8.67), n = 103) and abnormal motility group (total motility < 40% (24.2 ± 9.51), n = 102) (P = 0.0001) as presented in Table 1. No statistically significant differences in age (P = 0.088), pH (P = 0.055), semen volume (P = 0.48), motile sperm LIN (P = 0.14), and motile sperm BCF (P = 0.92) were observed between normal and abnormal motility groups. Motile sperm STR of the abnormal group (84.8 ± 8.05) showed slight reduction (P = 0.016)

Table 1
Demographic characteristics of semen parameters according to WHO reference values 2010.

Parameters	Total motility > 40% (n = 103)	Total motility < 40% (n = 102)	Percent of < 40%/ > 40%	FDR-corrected-P-value
Semen volume (ml)	4.07 ± 1.41	4.22 ± 1.62	103.7	0.5201
Sperm concentration (10^6/ml)	32.1 ± 20.1	9.3 ± 8.39	28.9	0.0004
Progressive motility (PR, %)	44.6 ± 8.39	19.6 ± 8.43	43.9	0.0004
Total motility (PR + NP, %)	55.6 ± 8.67	24.2 ± 9.51	43.52	0.0004
Motile sperm VCL (μm/s)	47.7 ± 6.67	42.0 ± 9.93	0.88	0.0016
Motile sperm VSL (μm/s)	33.1 ± 5.15	29.5 ± 7.43	0.89	0.0083
Motile sperm VAP (μm/s)	35.6 ± 5.22	31.8 ± 7.52	0.89	0.0062
Motile sperm BCF (Hz)	5.42 ± 0.482	5.40 ± 0.882	0.99	0.92
Motile sperm ALH (μm)	3.25 ± 0.802	2.75 ± 0.919	0.84	0.0044
Motile sperm LIN (%)	68.3 ± 5.84	65.6 ± 9.93	0.96	0.1654
Motile sperm STR (%)	89.9 ± 2.89	84.8 ± 8.05	0.94	0.026
Semen pH	7.38 ± 0.089	7.35 ± 0.133	99.6	0.0787
Age(years)	30.72 ± 4.89	31.84 ± 4.42	103.7	0.1141

compared with the normal group (89.9 ± 2.89). The levels of sperm concentration ($P = 0.0001$), progressive motility ($P = 0.0001$), motile sperm VCL ($P = 0.0005$), motile sperm VSL ($P = 0.0045$), motile sperm VAP ($P = 0.0029$), and motile sperm ALH ($P = 0.0017$) were significantly lower in the abnormal motility group relative to those of the normal motility group.

3.2. Ionomic profile of seminal plasma of normal and abnormal sperm motility groups

The difference in ionomic profile of seminal plasma of normal versus abnormal sperm motility groups was summarized in Table 2. Among 27 ions and their isotopes, 20 ions were reliably detected by ICP-MS. B, Cr, Pd, Ag, Sn, Pt and Hg were not detectable in the samples. The ionomic data fitted a normal distribution were analyzed based on SPSS 22.0 software. Notably, Ni levels were significantly decreased ($P = 0.00007$) in the abnormal motility group compared to the normal

Table 2
Determination of seminal plasma ion concentrations by ICP-MS. ND means not detected, ND-Sig.TM means normal distribution significance-total motility.

Ions(Unit)	Total motility > 40%	ND-Sig. TM > 40%	Total motility < 40%	ND-Sig. TM < 40%	Percent of < 40%/ > 40%	FDR-corrected P-value
Li / 7 (μg/L)	1.28 ± 490.8	0.200	1.25 ± 608.9	0.200	97.6	0.7018
B / 11 (μg/L)	ND	ND	ND	ND	ND	ND
Na / 23 (mg/L)	3288 ± 188.3	0.200	3314 ± 151.6	0.112	100.8	0.4483
Mg /24 (mg/L)	**140.1 ± 23.5**	**0.056**	**153.6 ± 25.7**	**0.200**	**109.6**	**0.0437**
P / 31 (mg/L)	1086 ± 106.4	0.200	1084 ± 118.3	0.061	98.0	0.7018
S / 34 (mg/L)	**296 ± 31.2**	**0.066**	**330 ± 50.7**	**0.106**	**111.5**	**0.0174**
K / 39 (mg/L)	1414 ± 174.2	0.078	1410 ± 185.2	0.200	99.7	0.7018
Ca / 40 (mg/L)	366.7 ± 58.7	0.080	371.3 ± 52.1	0.060	101.2	0.8679
Ca / 44 (mg/L)	280.4 ± 40.6	0.072	283.1 ± 40.8	0.200	100.9	0.8897
Ti / 47 (μg/L)	438.1 ± 35.7	0.080	464.3 ± 74.8	0.200	105.9	0.2065
Ti / 48 (μg/L)	1727.6 ± 246.8	0.121	1749.6 ± 242.2	0.200	101.3	0.8679
Cr / 52 (μg/L)	ND	ND	ND	ND	ND	ND
Cr / 53 (μg/L)	ND	ND	ND	ND	ND	ND
Mn /55 (μg/L)	7.57 ± 2.37	0.200	7.49 ± 2.07	0.200	98.9	0.8897
Fe / 56 (μg/L)	201.2 ± 90.2	0.071	181.6 ± 83.6	0.200	90.3	0.7018
Fe / 57 (μg/L)	1326 ± 480.2	0.200	1398 ± 390.8	0.159	105.4	0.7018
Ni / 58 (μg/L)	**10.22 ± 3.83**	**0.129**	**5.69 ± 1.93**	**0.200**	**55.7**	**0.00203**
Co / 59 (μg/L)	0.71 ± 0.15	0.200	0.74 ± 0.17	0.200	104.8	0.7081
Ni / 60 (μg/L)	**10.75 ± 3.86**	**0.121**	**6.55 ± 2.11**	**0.200**	**60.7**	**0.0058**
Cu / 63 (μg/L)	147.1 ± 44.9	0.200	153.8 ± 46.1	0.072	104.6	0.7091
Zn / 64(mg/L)	**160.7 ± 31.5**	**0.200**	**178.3 ± 35.3**	**0.200**	**110.9**	**0.0494**
Cu / 65 (μg/L)	151.9 ± 44.8	0.200	159.6 ± 45.9	0.056	105.1	0.7018
Zn / 66(mg/L)	**164.3 ± 32.3**	**0.200**	**181.9 ± 36.5**	**0.200**	**110.7**	**0.0494**
As / 75 (μg/L)	**2.86 ± 0.74**	**0.200**	**3.81 ± 1.20**	**0.200**	**132.9**	**0.0226**
Se / 78 (μg/L)	**49.6 ± 12.3**	**0.135**	**55.1 ± 18.5**	**0.081**	**110.9**	**0.0195**
Sr / 88 (μg/L)	102.9 ± 19.6	0.108	105.0 ± 16.9	0.113	102.0	0.7216
Mo / 95 (μg/L)	1.17 ± 0.54	0.050	1.18 ± 0.53	0.200	101.5	0.7018
Mo / 98 (μg/L)	1.27 ± 0.53	0.200	1.24 ± 0.51	0.200	97.5	0.7018
Pd / 105(μg/L)	ND	ND	ND	ND	ND	ND
Pd / 106(μg/L)	ND	ND	ND	ND	ND	ND
Ag /107(μg/L)	ND	ND	ND	ND	ND	ND
Ag /109(μg/L)	ND	ND	ND	ND	ND	ND
Cd /111(μg/L)	0.17 ± 0.078	0.200	0.19 ± 0.068	0.200	111.8	0.8897
Cd /112(μg/L)	0.097 ± 0.051	0.088	0.071 ± 0.053	0.200	73.2	0.7018
Sn / 118(μg/L)	ND	ND	ND	ND	ND	ND
Sn / 120(μg/L)	ND	ND	ND	ND	ND	ND
Pt / 194 (μg/L)	ND	ND	ND	ND	ND	ND
Pt /195 (μg/L)	ND	ND	ND	ND	ND	ND
Hg /201(μg/L)	ND	ND	ND	ND	ND	ND
Pb / 206(μg/L)	2.28 ± 2.17	0.080	2.43 ± 2.09	0.071	106.6	0.8897
Pb / 208(μg/L)	2.11 ± 1.98	0.069	2.02 ± 1.93	0.095	95.7	0.8897

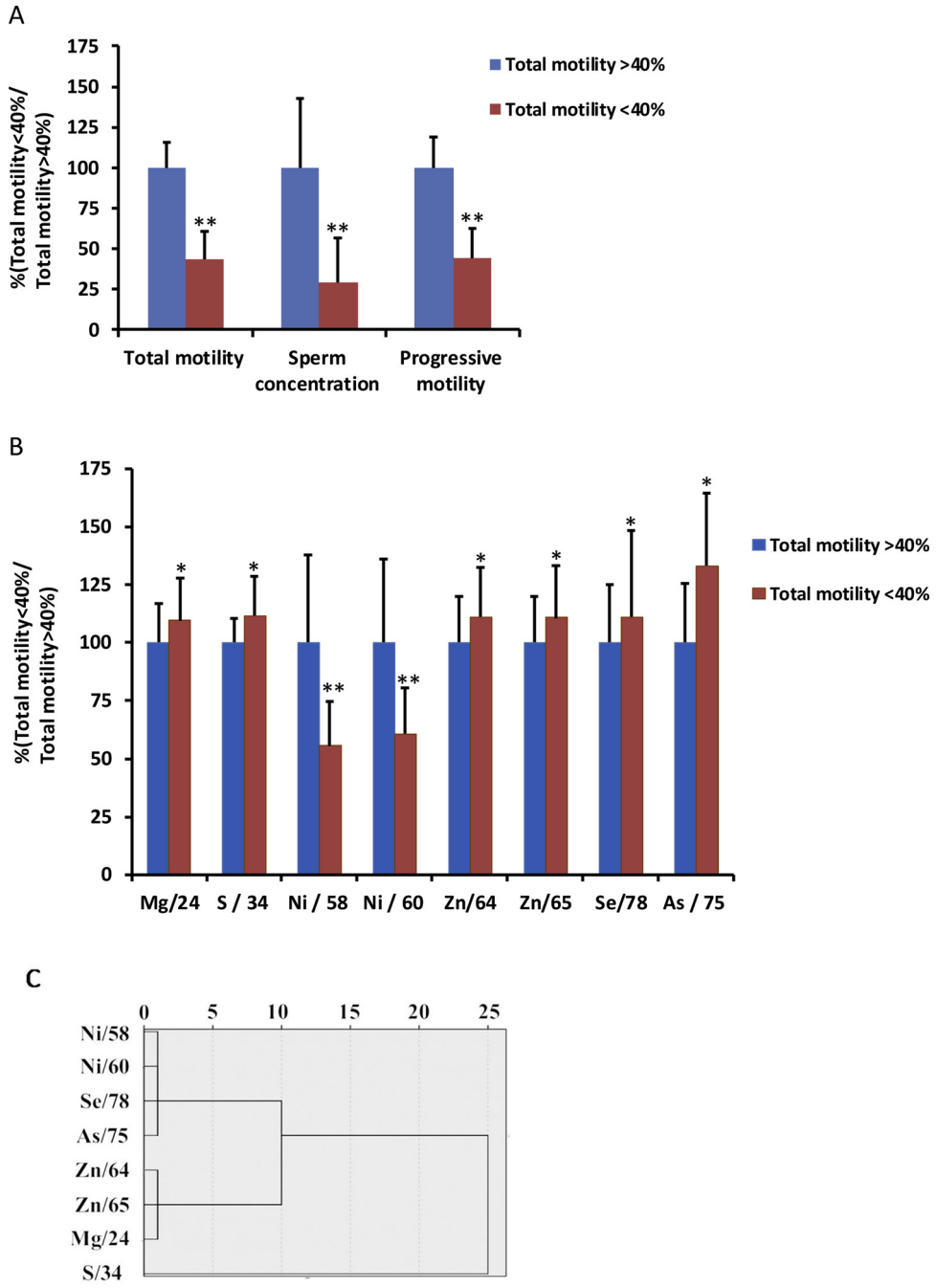

Fig. 1. The correlation between S, Mg, Se, Zn, As, Ni and sperm quality.
(A) The correlation between total motility, sperm concentration, and progressive motility.
(B) Comparison of elements contents between normal group and abnormal group.
(C) Related ions identification by clustering analysis.
Asterisk* indicates significant difference from abnormal group control (* $p < 0.05$, ** $p < 0.01$).

motility group. As levels of the abnormal group are 132.9% relative to those of the normal group ($P = 0.0039$). Meanwhile, the concentration of S was significantly higher in the abnormal group ($P = 0.0018$). A moderate correlation of Mg ($P = 0.019$) and Zn ($P = 0.03$) was also observed. No significant change in the other 15 ions, such as P, Fe and Cu was found. Patient age was not correlated with any of the seminal plasma ions (data not shown), which was consistent to previous studies.

3.3. Correlations between seminal ion concentrations and semen parameters

The associations between sperm quality parameters and seminal ion concentrations were shown in Fig. 1. The correlation between ion concentrations and semen parameters was calculated by Excel correlation analysis and all correlation coefficients were more than 0.98. Sperm concentration and progressive motility were significantly lower in the abnormal group, which was consistent to total motility (Fig. 1A). Notably, we found that Ni was significantly associated with the sperm motility abnormal group (Fig. 1B, $P = 0.002$). S, Se, As, Mg and Zn ions were increased in the abnormal group compared with those of the normal group. However, we found that most ions (e.g., Li, Na, P, Ca, Ti, Cr, Mn, Fe, Co, Cu, Sr, Mo, Cd and Pb) were not associated with sperm concentration, progressive motility, and total motility. The clustering

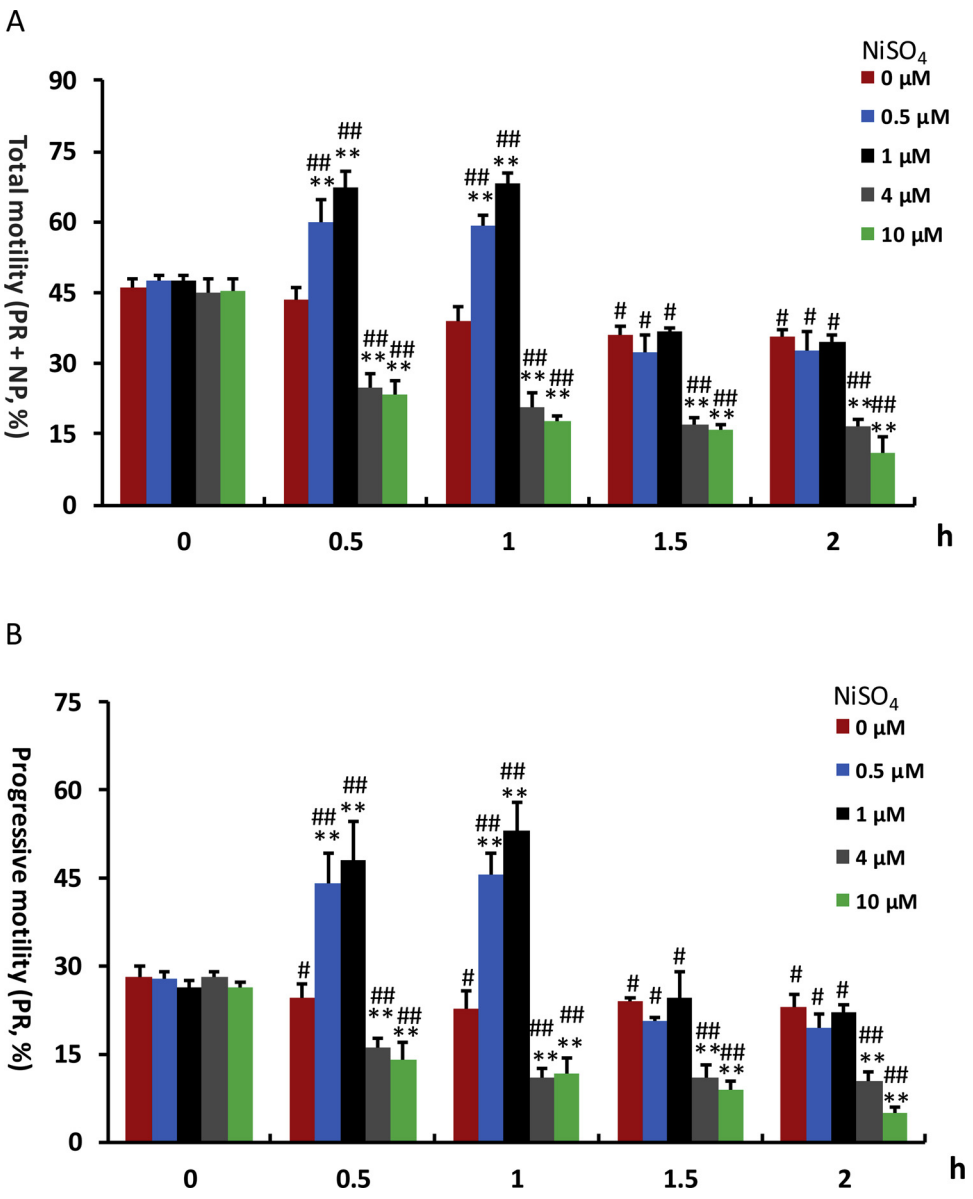

Fig. 2. Time course and concentration effects of Ni^{2+} on sperm total motility and progressive motility.
The abnormal semen samples (total motility < 40%) were centrifuged at 1000 rpm for 5 min to collect low activity sperms and diluted to 20×10^6/ml. Low activity sperm were incubated with 0, 0.5, 1, 4 and 10 μM Ni^{2+} for 0, 0.5, 1, 1.5 and 2 h, respectively. Control sperms were incubated with saline. Sperm total motility (A) and progressive motility (B) were measured and expressed as a percentage. Data presented are mean ± SD of five samples per point. Asterisk* indicates significant difference from concentration course control (* $p < 0.05$, ** $p < 0.01$). Pound # means significant difference from time course control (# $p < 0.05$, # # $p < 0.01$).

analysis indicated the relationships of Ni, Se, As, Zn, Mg and S, especially for Ni, Se and As (Fig. 1C). Collectively, our data suggests that Ni, As and Se levels in seminal plasma exhibited strong correlations with total motility, concentration and progressive motility of sperm.

3.4. Ni^{2+} increases sperm total motility and progressive motility in vitro

Given the fact that Ni levels were much lower in the abnormal motility group compared to the normal motility group, we hypothesized additional Ni may promote sperm total motility and progressive motility. To address this, low activity sperms (total motility < 40%) were separated and diluted to 20×10^6/ml. 50 μl diluted sperm samples were incubated for 0.5, 1, 1.5, or 2 h in flasks without (control) or with indicated $NiSO_4$ (0.5, 1, 4, and 10 μM). Both total sperm motility (Fig. 2A) and progressive motility (Fig. 2B) increased significantly in low activity samples treated with 0.5 and 1 μM $NiSO_4$ incubated for 0.5 and 1 h. Compared with the control sample (0 μM $NiSO_4$), sperm motility and progressive motility treated with 1 μM $NiSO_4$ incubated for 1 h are up to 143.4% (Fig. 2A) and 201.9% (Fig. 2B), respectively. When extending the incubation time to 1.5 and 2 h, total sperm motility and progressive motility slightly decreased but significantly. High Ni concentration treatments such as 4 and 10 μM dramatically decreased total sperm motility and progressive motility (Fig. 2A and B). Collectively, these data suggest that low concentration of Ni increases sperm total motility and progressive motility in vitro.

3.5. Ni^{2+} promotes eukaryotic yeast cell growth

Because our data show low concentration of Ni increases sperm total motility and progressive motility in vitro, we proposed that low concentration of Ni is also benefical for the other eukaryotic cells. To support this, we used *Saccharomyces cerevisiae* as a eukaryotic cell model as most yeast cell genes are conserved in mammalian cells. Exponentially growing cells were diluted (A600 = 0.1), and then cells (5 μl) were spotted on glucose media (YPD) supplemented with indicated concentrations of Ni ($NiSO_4$). As can be seen in Fig. 3A, the addition of 1and 2 μM $NiSO_4$ in the YPD medium promoted yeast cell growth up to 151% and 153% (Fig. 3B), respectively. The high concentration Ni (> 5 μM) displayed no effect on the yeast cells (Fig. 3A). Therefore, these results indicate that low concentration of Ni contributes to eukaryotic yeast cell growth.

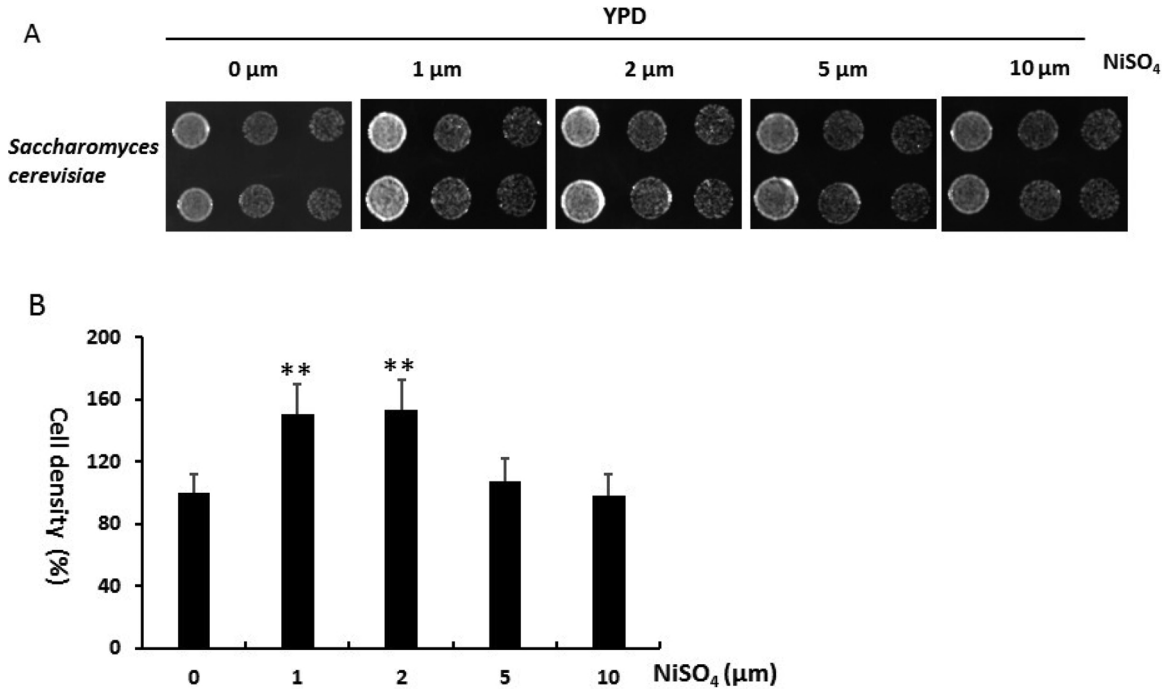

Fig. 3. Low concentration Ni^{2+} improves yeast cell growth in YPD media.
Yeast *Saccharomyces cerevisiae* cells were cultured in glucose media (YPD) and spotted on solid YPD media that was supplemented indicated $NiSO_4$. Cell growth was monitored for 2 days and photographed (A). Five repeats for each treatment and quantitated (B). Asterisk* indicates significant difference from control (* $p < 0.05$, ** $p < 0.01$).

4. Discussion

It is becoming increasingly important to understand male infertility and discover novel diagnostic and therapeutic methods since six percent of adult males are thought to be infertile [7]. In the present study, to assess the association between ion concentrations of seminal plasma and human sperm quality, a total of 205 semen samples were collected from the most developed Yangtze River Delta Region, in eastern China were divided into normal group (> 40%, n = 102) and abnormal group (< 40%, n = 103) depending on total motility. According to this classification, we observed significant correlations between total motility and other semen parameters (sperm concentration, progressive motility, motile sperm, motile sperm VSL, motile sperm VAP, motile sperm ALH and motile sperm STR, Table 1), especially sperm concentration and progressive motility (Fig. 1A).

Sperm quality is completely different depending on geographical location and environmental factors [26,28–30]. Jeng et al measured metal levels in both seminal plasma and urine from Taiwan by atomic absorption spectrometry [19]. In our study, first, seminal samples were collected from the most developed and thriving sector of eastern China, whose geographical location and environment are totally different from the Taiwan's study. Second, inductively coupled plasma mass spectrometry (ICP-MS) is used in our study to measure ion concentration, which is more sensitive than atomic absorption spectrometry. Third, ionomics was conducted in this study rather than metallomics. Owing to the above differences, our results revealed novel findings compared to previous reports [19].

The present study measured 27 seminal plasma ions and their isotopes of which 20 ions were reliably detected by the ICP-MS analysis. B, Cr, Pd, Ag, Sn, Pt and Hg were undetectable in seminal plasma samples due to their trace concentrations. The heavy metal Ni ($P = 0.002$) was significantly decreased in the abnormal motility group compared to the normal motility group. The concentrations of the non-essential element arsenic ($P = 0.0226$) and non-metal ion S ($P = 0.0174$) significantly increased in the abnormal group. Mild associations from Mg ($P = 0.0437$) and Zn ($P = 0.0494$) were also observed. Normally, the Zn and Mg transporters also transport Ni via non-specific uptake. High contents of Zn and Mg in the abnormal group may reduce Ni level owing to competition uptake by these transporters. Meanwhile, the other 15 ions, such as P, Fe and Cu had no significant changes. The ionomics of seminal plasma provides a comprehensive profile to evaluate seminal quality and a more accurate assessment.

Recent studies have shown that older men have significantly higher levels of sulfur in their seminal plasma and that seminal plasma sulfur was negatively associated with sperm motility and structural aberrations and positively associated with DNA fragmentation [36]. De Rosa et al. reported concentrations of sulphur oxides in men occupationally exposed to environmental pollutants (sperm total motility: 34.7%) were significantly higher than those of control men randomly selected (sperm total motility: 56.8%) [37]. These findings are consistent with our results that indicate sulfur content is obviously higher in men with low sperm total motility.

Magnesium (Mg) is an important cation found in many enzymatic systems. Magnesium may play a role in spermatogenesis, especially in sperm motility. Also, Mg is regarded as a marker of the secretions of the seminal vesicles and acts as an intracellular calcium antagonist [38]. The prior studies explored that higher levels of Mg in the seminal plasma of fertile males are not statistically associated with decreased motility [38,39]. However, an association was found between high Mg concentrations and low linearity of sperm movements as expressed by a decrease in LIN [12]. We observed a significant correlation between high Mg concentrations of semen plasma and low sperm concentration and motility.

Selenium (Se) is a trace element essential for normal spermatogenesis of mammals and plays a critical role in antioxidant defense. Se in seminal plasma correlates with good spermatozoa concentrations, motility, and morphology [40,41]. Some research groups reported that a slight or significant increase in selenium concentration was associated with low sperm motility [19,39,42], which confirmed our findings that Se is necessary for semen quality, but high Se is not associated with

high semen quality.

Zinc (Zn) is widely involved in male reproduction and fertility [36,38,43,44] and we observed that the abnormal motility group had increased seminal zinc. The previous studies also indicated high Zn concentrations in the low sperm motility groups [19,39]. Mg, Se and Zn are essential elements for normal spermatogenesis of mammals but in our current studies all these elements exhibited high contents in the abnormal motility group. These results suggest there is a defect in acquisition or utilization of these metal ions for the abnormal motility sperm and causes high concentrations in the semen.

Arsenic (As) is a nonessential and toxic element for humans due to As-induced oxidative stress [45]. Previous studies found that As in seminal plasma or blood is associated with decreased semen quality [46–48]. In the present study, we found that As in seminal plasma was significantly associated with declined sperm quality. Similar results were observed in some previous studies that used urine and seminal plasma as targets [47,48]. Shen et al. showed that elevated urinary concentration of As is significantly associated with male infertility [48]. In another studies, Sengupta et al. clearly suggested that there is a direct correlation between the presence of arsenic in drinking water (and thus in the seminal plasma) and incidence of male infertility [47]. SO_x and Arsenic compounds are main and highly toxic environmental pollutants attributed to the impairment of semen quality. In our study, the abnormal group may be exposed to SO_x and Arsenic compounds and accumulation of these chemicals leads to toxicity to the sperm activity.

In our current seminal plasma ionomics, Nickel (Ni) is the only element significantly reduced in the low sperm activity group. It is supposed that Ni deficiency is a factor reducing sperm activity and this is the reason why we selected Ni for further experimental verification. Ni is an essential component of several metallo-enzymes involved in energy and nitrogen metabolism from prokaryotes to eukaryotes, but Ni-containing proteins or compounds have not been described in any mammal including humans [49]. Early studies on nickel essentiality with experimental animals indicated that nickel deprivation diminished sperm density and motility in the epididymis and sperm production rate [50,51]. One possible mechanism through which nickel could affect sperm motility is by altering cyclic nucleotide gated channels (CNG) functions which are important in sperm physiology [50]. Also, Ni functions in humans or animals either as a structural component in specific metalloenzymes or as a cofactor facilitating the intestinal absorption of the ferric ion [51] and Ni deficiency also significantly decreased the weight of the seminal vesicles and prostate glands [52]. The in vitro effect of nickel on bovine spermatozoa suggested low concentration Ni in short time periods of culture stimulated spermatozoa motility [53]. As with other essential elements, excess nickel also impairs reproductive performance in animals [51,54] owing to Ni-induced oxidative stress [55].

Above 85% of infertile males can actually produce sperms with abnormal functions suggesting that except for sperm itself, the seminal plasma, microenvironment of sperm, could contribute to male fertility [10,56]. In agreement with previous findings, we found low Ni concentration of seminal plasma in the poor sperm quality group in humans. To further address Ni as an important metal ion affecting seminal plasma microenvironment, our in vitro effects of Ni assay proved low concentration of Ni in short time periods (0.5–1 h) of culture stimulated human sperm total motility and progressive motility. In addition, we elucidated low concentration of Ni is beneficial for eukaryotic yeast cell growth which confirmed Ni is essential from the cell growth side. In normal condition, the bio-available nickel is deficiency due to nickel is a trace element. Maybe this is the reason why supplied low concentration nickel to the medium promoting yeast cell growth due to improving cell energy and nitrogen metabolism. In our abnormal group, there may be defects in Ni uptake or dietary Ni deprivation leading to low Ni concentration in the seminal plasma.

5. Conclusions

In conclusion, our study revealed a strong correlation between S, Mg, Se, Zn, As, Ni and seminal quality. For the first time we discovered Ni increases human sperm total motility and progressive motility as well as promotes eukaryotic cell growth. This finding will open a new potential avenue for assessment and improvement of sperm quality.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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