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RESEARCH ARTICLE

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Metabolomic characterization of semen from asthenozoospermic patients using ultra-high-performance liquid chromatography–tandem quadrupole time-of-flight mass spectrometry

Li Li[1] | Xinrui Hao[1] | Hua Chen[2] | Liyuan Wang[2] | Aolei Chen[2] | Xiaotian Song[2] | Zhiyan Hu[1] | Ying Su[1] | Han Lin[1] | Pei Fan[3]

[1]Zhejiang Provincial Key Laboratory of Anesthesiology, Department of Anesthesiology, the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

[2]Reproductive Health Centre, Department of Obstetrics and Gynecology, the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

[3]Zhejiang Provincial Key Laboratory of Orthopedics, Department of Orthopedics, the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence
Pei Fan, Zhejiang Provincial Key Laboratory of Orthopedics, Department of Orthopedics, the Second Affiliated Hospital of Wenzhou Medical University, Number 109, Xueyuan West Road, Wenzhou 325027, Zhejiang, China.
Email: fanpei@wmu.edu.cn

Han Lin, Zhejiang Provincial Key Laboratory of Anesthesiology, Department of Anesthesiology, the Second Affiliated Hospital of Wenzhou Medical University, Number 109, Xueyuan West Road, Wenzhou 325027, Zhejiang, China.
Email: nanlinhannansh@qq.com

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Abstract

Asthenozoospermia (AS) is a common factor of male infertility, and its pathogenesis remains unclear. The purpose of this study was to investigate the differential seminal plasma metabolic pattern in asthenozoospermic men and to identify potential biomarkers in relation to spermatogenic dysfunction using sensitive ultra-high-performance liquid chromatography–tandem quadruple time-of-flight MS (UHPLC–Q-TOF/MS). The samples of seminal plasma from patients with AS ($n = 20$) and healthy controls ($n = 20$) were checked and differentiated by UHPLC–Q-TOF/MS. Compared with the control group, the AS group showed a total of nine significantly different metabolites, including increases in creatinine, uric acid, N^6-methyladenosine (m^6A), uridine, and taurine and decreases in carnitine, nicotinamide, *N*-acetylputrescine and L-palmitoylcarnitine. By analyzing the correlation among these metabolites and clinical computer-assisted semen analysis reports, we found that m^6A is significantly correlated with not only the four decreased metabolites but also with sperm count, motility, and curvilinear velocity. Furthermore, nicotinamide was shown to correlate with other identified metabolites, indicating its important role in the metabolic pathway of AS. Current results implied that sensitive untargeted seminal plasma metabolomics could identify distinct metabolic patterns of AS and would help clinicians by offering novel cues for discovering the pathogenesis of male infertility.

KEYWORDS

asthenozoospermia, N^6-methyladenosine, nicotinamide, UHPLC–Q-TOF/MS

Li Li, and Xinrui Hao. These authors contributed equally to this work.

1 | INTRODUCTION

Infertility has become a serious health problem, influencing approximately 10% of reproductive-age couples, with half of these infertility cases attributed to male factors (Practice Committee of the American Society for Reproductive Medicine, 2015). Asthenozoospermia (AS) is a common cause of male infertility characterized by reduced forward motility or absence of sperm motility in fresh ejaculate (Jodar, Kalko, Castillo, Ballesca, & Oliva, 2012). Routine semen analysis may indicate defective sperm production or weakened sperm motility. However, the etiologies of male infertility are still uncertain, and a full understanding of AS remains a challenge.

Increasing evidence has shown that abnormal metabolic phenotypes in body fluids reflect the pathogenesis and pathophysiology of disease (Huang et al., 2013; Kalia & Costa, 2015; Liu et al., 2019; Zhang et al., 2014). Seminal plasma is one of these body fluids and a good sample for metabolomic analysis in the evaluation of male fertility/infertility. Any alterations at the molecular level in spermatozoa and seminal plasma may affect male fertility (Milardi, Grande, Vincenzoni, Castagnola, & Marana, 2013; Wang et al., 2009). Therefore, combining metabolomics studies with seminal plasma analysis would be helpful for studies of male infertility.

Various techniques, such as nuclear magnetic resonance (NMR), MS, Fourier transform infrared spectroscopy and Raman spectroscopy, have been developed for the study of metabolomics. NMR spectroscopy has the advantages of simple preparation and non-destruction of samples and has been widely used in metabolomics. However, compared with NMR, MS exhibits ultra-high sensitivity and broad dynamic range and requires low metabolite concentrations in samples. Therefore, MS-based metabolomics has become the most popular metabolomics strategy (Kim, Kim, Kim, Hwang, & Yoo, 2016; Paiva et al., 2015; Zhao et al., 2018).

Considering the aforementioned factors, MS-based metabolomics, especially with ultra-high-performance liquid chromatography–tandem quadrupole time-of-flight MS (UHPLC–Q-TOF/MS), would be able to detect small variations in metabolites between asthenozoospermic patients and healthy controls (Zhu et al., 2016), although there have been two studies of seminal plasma metabolomics using NMR (Gilany, Moazeni-Pourasil, Jafarzadeh, & Savadi-Shiraz, 2014; Zhang, Diao, Zhu, Li, & Cai, 2015). To our knowledge, this is the first report of a human seminal plasma metabolomics study using UHPLC–Q-TOF/MS. The aim of this study was to define whether seminal plasma metabolomics analysis could provide new insights into the etiology of AS and whether the identified molecular markers could serve as potential biomarkers that may help clinicians diagnose and treat AS.

2 | MATERIALS AND METHODS

2.1 | Study design and participant recruitment

The study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University (SAHWMU; 2016–0054) and conducted on the basis of the Helsinki Declaration. Each participant was fully informed of the aim of the study and signed the informed consent form. To remove the influence of confounders that may interfere with metabolism, participants with any of the following criteria were excluded: (1) current reproductive system disease, such as malformation, trauma, tumor or infection; (2) any history of varicocele, cryptorchidism, orchitis, epididymitis and/or vas deferens or ejaculatory duct obstruction; (3) chronic systemic metabolic disorder, such as diabetes or hepatic disease, or occupational exposure and (4) chromosome abnormality, including Y chromosome microdeletions and other known factors related to male infertility and metabolism.

2.2 | Semen collection

The participants were instructed to collect the specimens into specific containers following 3–5 days of sexual abstinence. After complete liquefaction at 37°C for 30 min, parameters including semen volume, sperm concentration, sperm motility and viability were analyzed by a computer-assisted semen analysis (CASA) system under 200× magnification. Sperm morphology was assessed by a SpermFunc Diff-Quik staining kit according to the manufacturer's manual (BRED Life Sciences). As healthy controls, we randomly selected 20 men with a proven normal CASA test result as assessed by the World Health Organization Manual for Semen Analysis (2010) [Total motility (progressive motility + non-progressive motility, %) ≥ 40; sperm concentration (10^6 ml^{-1}) ≥ 15; sperm morphology (normal forms, %) ≥ 4]; as the treatment group, we included 20 asthenozoospermic men referred to the Reproductive Center of SAHWMU for infertility treatment. The main criterion for the classification of asthenozoospermic men was low sperm motility (<40% motile spermatozoa; >15 million spermatozoa/ml; >4% normal forms). After analysis, the remaining human seminal plasma was purified by centrifugation of semen (825× *g*, 10 min, 25°C), and then the supernatant (seminal plasma) was collected and stored at −80°C until further metabolite extraction.

2.3 | Metabolite extraction and derivatization for UHPLC–Q-TOF/MS

All the seminal plasma samples were thawed at 4°C and homogenized using a vortex mixer. Then, each sample was treated according to the procedure described by Liu et al. (2019). In brief, 100 μl of sample was removed and transferred to Eppendorf tubes mixed with 400 μl of methanol (106035, Merck, Germany)/acetonitrile (101164, Merck, Germany) (1:1, v/v). After vortexing for 30 s, the tubes were incubated at −20°C for 10 min and then centrifuged at 14,000× *g* for 15 min at 4°C, followed by collection of supernatants and drying with nitrogen. The lyophilized powder was stored at −80°C until further analysis.

Before analysis, the lyophilized samples were thawed at 4°C and dissolved in 100 μl solvent mixture containing water/acetonitrile (5:5,

v/v). Then, the samples were vortexed for 1 min and centrifuged at 14,000× *g* for 15 min at 4°C. The supernatants were limited to UHPLC–Q-TOF/MS analysis. Pooled quality control (QC) samples were also prepared by mixing equal amounts (30 μl) of each sample. The QC samples were utilized to monitor the UHPLC–Q-TOF/MS response in real time (Liu et al., 2019).

2.4 | UHPLC–Q-TOF/MS analysis

Metabolic analysis of seminal plasma samples was performed on an Agilent 1290 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) coupled with an AB SCIEX Triple TOF 6600 System (Framingham, MA, USA; Wang et al., 2017). Chromatographic separation was performed on ACQUITY HSS T3 1.8 μm (2.1 × 100 mm) columns (Waters, Milford, MA, USA) with both positive and negative modes. The column temperature was set at 25°C for operation. The mobile phases of 0.1% formic acid in water and 0.1% formic acid (100264, Merck, Germany) in acetonitrile (A) were used in positive ionization mode, whereas 0.5 mM ammonium fluoride (101164, Merck, Germany) in water or in acetonitrile (B) was used in negative ionization mode. In the positive (negative) mode, the elution gradient initially began with 1% A (B) for 1 min and linearly increased to 100% A (B) at 8 min, followed by maintenance for 2 min, and then returned to 1% A (B) for approximately 2 min of equilibrium. The delivery flow rate was set at 300 μl/min, and a 2 μl aliquot of each sample was injected onto the column. TOF/MS was also performed in both ionization modes. Electrospray ionization source conditions on the triple TOF system were similar to those in the literature (Liu et al., 2019) and set as follows: ion source gas 1, 40 psi; ion source gas 2, 60 psi; curtain gas, 30 psi; source temperature, 650°C; ion spray voltage floating, 5000 V (+) and −4500 V (−). Information-dependent acquisition, an artificial intelligence-based product ion scan mode, was used to detect and identify the tandem mass spectrometry spectra. The parameters were set as follows: declustering potential, 60 V (+) and −60 V (−); collision energy, 50 V (+) and −20 V (−); excluding isotopes within 4 Da; candidate ions to monitor per cycle, 10. The analysis process was conducted with the assistance of Applied Protein Technology Co., Ltd. (Shanghai, China).

2.5 | Data analysis

The raw data generated by UHPLC–Q-TOF/MS were first converted into mzML format files by the ProteoWizard MS converter tool, and then data processing was accomplished using the XCMS online software (https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage), including non-linear alignment in the time domain, automatic integration, extraction of the peak intensities and data filtering. MetaboAnalyst 4.0 (http://www.metaboanalyst.ca) was used for the statistical analysis (Chong et al., 2018). Principal component analysis was performed, and a heat map of hierarchical clustering analysis was generated for the unsupervised multivariate statistical analysis. Partial least squares-discrimination analysis (PLS-DA) was conducted as a supervised method to identify the important variables with discriminative power. Orthogonal partial least squares-discriminant analysis (OPLS-DA) is a supervised pattern-recognition procedure that combines the existing theory of PLS-DA and orthogonal signal correction. The OPLS-DA model removes variability not relevant to group separation, and normally, only one predictive component is used for the discrimination between two classes. The PLS-DA and OPLS-DA models were validated based on the multiple correlation coefficients (R^2) and cross-validated R^2 (Q2) in cross-validation and permutation tests by applying 2000 iterations ($p > .001$). By calculation of the variable importance in projection (VIP) score (>1) from the OPLS-DA model, a Student's *t*-test could be used to evaluate the significance of the biomarkers with a *p* value less than .05 as a significant metabolite.

Correlation coefficients were determined by the Spearman rank correlation test using two-tailed *p* values. The correlation plot was generated using the R package (version R 3.5.3; R Core Team, Vienna, Austria); $p < .05$ was set as a significant difference.

3 | RESULTS

3.1 | QC of UHPLC–Q-TOF/MS analysis of the seminal plasma samples

The general semen characteristics of the two groups of participants are presented in Table 1. A typical UHPLC–Q-TOF/MS total ion chromatogram derived from seminal plasma metabolic profiling of the QC samples is shown in Figure 1a. The retention time and response intensity of the QC sample's mass spectrum peak were appropriate, which demonstrated that the analytical method (including the pre-processing method and instrumental analysis) was stable and reliable. Representative UHPLC–Q-TOF/MS total ion chromatograms of seminal plasma samples from the asthenozoospermic group and the healthy control group are displayed in Figure 1b,c, respectively. In total, 3060 molecular features were extracted from each sample using the XCMS software, and the peak intensity of each feature was obtained.

3.2 | Untargeted metabolomics analysis of seminal plasma obtained from study participants

To identify ion peaks that could be used to differentiate between the metabolite profiles of asthenozoospermic patients and healthy controls, we used subsequent analysis models to determine the best fit that could potentially reflect the class discriminating variation. First, we plotted the unsupervised principal component analysis scores of all seminal plasma profiles from the asthenozoospermic and healthy control groups. However, the results did not show intrinsic clustering related to AS in the first two PCs, and the healthy control samples were dispersed among the AS groups (Figure 2a). Then, the PLS-DA model improved the discrimination between the two groups, and a reasonably good discrimination of asthenozoospermic patients from

TABLE 1 Semen characteristics of the healthy controls and asthenozoospermic patients subjected to UHPLC–Q-TOF/MS analysis

Group	N	Age (years)	Seminal volume (ml)	Sperm count (million/ml)	Total sperm motility (%)	Sperm with normal morphology (%)
Control	20	31.7 ± 0.8	3.22 ± 0.22	88.57 ± 9.02	74.75 ± 2.25	37.4 ± 2.83
Asthenozoospermia	20	32.65 ± 1.23	3.18 ± 0.89	47.75 ± 5.53**	28.95 ± 2.78**	24.52 ± 2.18*

*$p < .05$.
**$p < .01$.

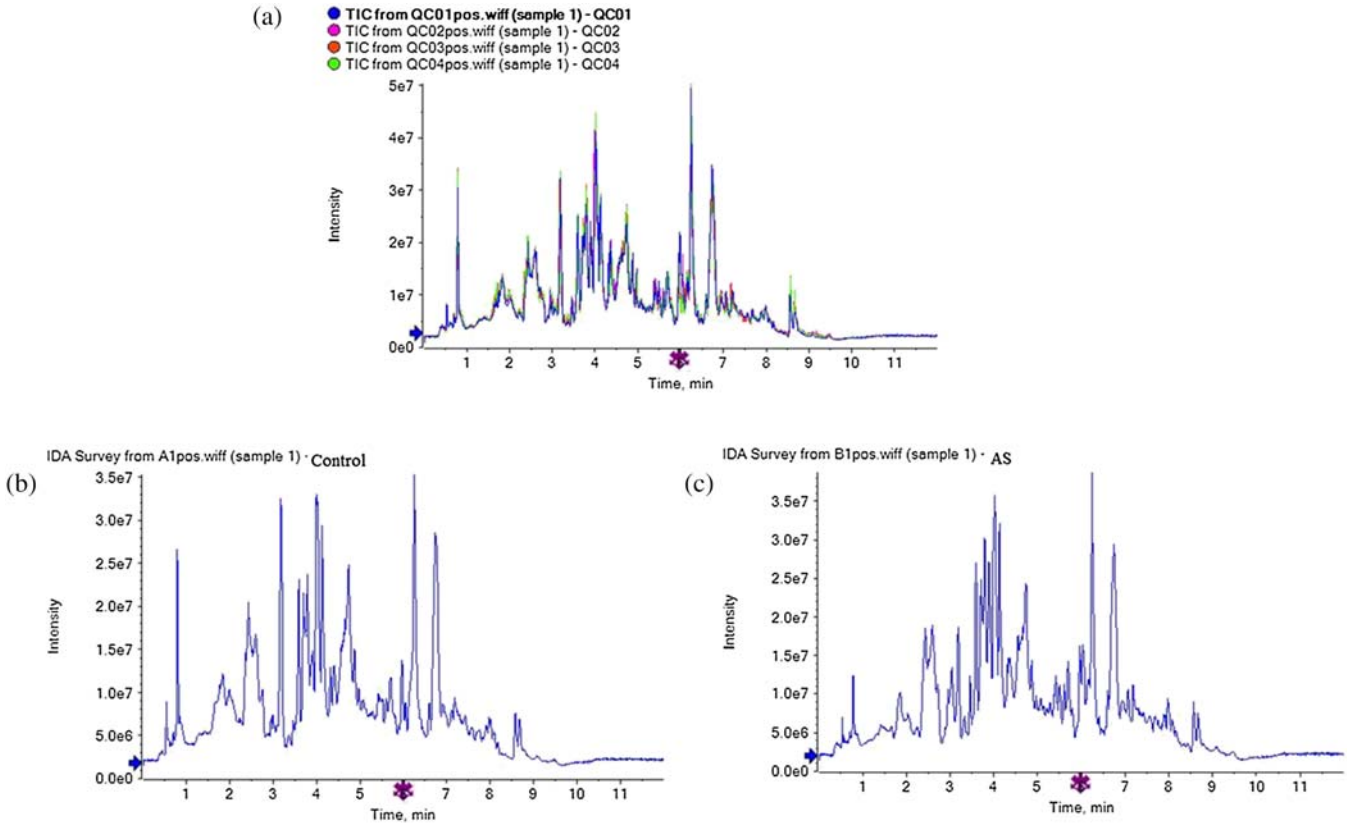

FIGURE 1 Representative base peak intensity chromatographic profiles of (a) quality control samples, (b) healthy controls, and (c) asthenozoospermic patients. TIC, total ion chromatogram

the healthy controls was obtained (Figure 2b). The cumulative R2Y and Q2Y were 0.731 and –0.0763, respectively. Further clearer class discrimination was obtained by the OPLS-DA model. As illustrated by the OPLS-DA score plot (Figure 2c), the R2Y and Q2Y were 0.67 and –0.411, respectively. As indicated in Figure 2d, permutation tests showed that the model is robust and not over-fitted (200 times, intercepts: R2 0.0, 0.67; Q2 0.0, –0.411). The validation plots strongly assured that the original (O)PLS-DA models were valid because both the permuted R2 and Q2 (cum) values to the left are lower than the original point to the right, and the Q2(cum) regression lines have a negative intercept.

3.3 | Identification of differential seminal plasma metabolites

On the basis of successful discrimination of the control and AS groups, the search for the specific metabolites that contributed to the metabolomic differences between the two groups was conducted. First, an OPLS-DA Volcano plot was analyzed to select the significantly differentially expressed metabolites between the AS and control groups (Supplementary figure 1). The hierarchical cluster analysis heat map of potentially significant metabolites with fold change analysis >1.5 and a Student's *t*-test ($p < .05$) is shown in Figure 3. Second, VIP was used to evaluate the influence of the expression pattern of each metabolite on the classification and discrimination among samples, and more than one VIP was selected for subsequent analysis. Then, a Student's *t*-test ($p < .05$) was applied to test the significance of these differential metabolites. Finally, we identified nine significantly different metabolite components between these two groups, as listed in Table 2. Among them, creatinine, uric acid, N^6-methyladenosine (m^6A), uridine and taurine were significantly elevated in asthenozoospermic patients compared with healthy controls, with increases of 2.532, 1.524, 1.4788, 1.3673 and 1.3023 times, respectively. In addition, carnitine, nicotinamide, *N*-acetylputrescine and

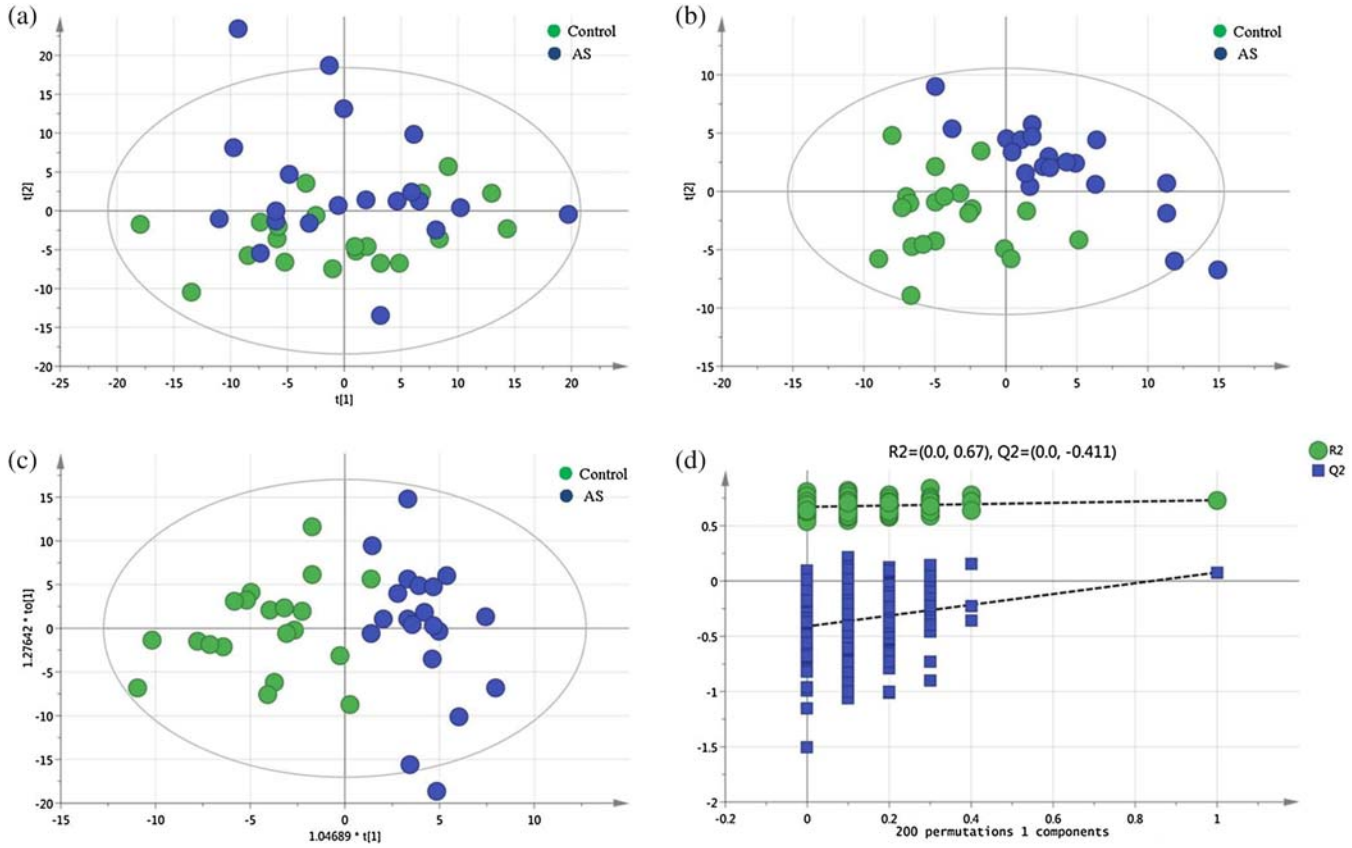

FIGURE 2 Multivariate statistical analysis of seminal plasma metabolic profiling in asthenozoospermic (AS) patients and healthy controls. (a) Principal component analysis score plot; (b) partial least squares-discriminant analysis score plot; (c) orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot; (d) statistical validation of the established OPLS-DA model with permutation analysis (200 random permutations)

palmitoylcarnitine were significantly reduced by 0.7959, 0.72726, 0.5798 and 0.5536 times, respectively. Through further correlation analysis of differential metabolites, a heat map was constructed (Figure 4), which clearly shows that m^6A was significantly negatively correlated with the other four decreased metabolites. Moreover, nicotinamide was significantly correlated with most of the differential metabolites, which implied its important role in AS.

3.4 | Correlation between seminal plasma metabolite and computer-assisted semen analyses

It is not clear how the current detected differentially metabolites would correlate with routine clinical reports, such as CASA. Therefore, we checked the correlation between the relative amount of significant seminal plasma metabolites and clinical CASA results. For this part, all of the data were included. As shown in Table 3, uric acid, nicotinamide and palmitoylcarnitine had significant correlations with all six parameters, whereas creatinine was only correlated with sperm motility. In addition, all of the identified metabolites were correlated with sperm motility. For the sperm concentration, an increase in m^6A had a significant negative correlation, whereas decreases in L-carnitine, nicotinamide and palmitoylcarnitine were positively correlated. Among the kinematic parameters of sperm motility, except creatinine and L-carnitine, all parameters were significantly correlated with curvilinear velocity, average path velocity and amplitude of lateral head displacement.

4 | DISCUSSION

In this study, with the improved, more sensitive UHPLC–Q-TOF/MS technology, we investigated the difference in seminal metabolomics patterns between asthenozoospermic patients and healthy controls. Our results showed unique seminal metabolomic features in the AS patients compared with the controls, and a total of nine metabolites were identified. m^6A was significantly correlated with not only the four decreased metabolites but also with sperm count, motility and curvilinear velocity. Furthermore, nicotinamide was shown to correlate with other identified metabolites and may serve as a supplemental therapy for clinical AS. Compared with other studies examining serum or urinary samples (Zhang et al., 2014, 2017) between asthenozoospermic patients and healthy controls, our study shows that seminal plasma is optimal for illustrating microenvironmental metabolic changes in the process of spermatogenesis with little interference from systemic actions.

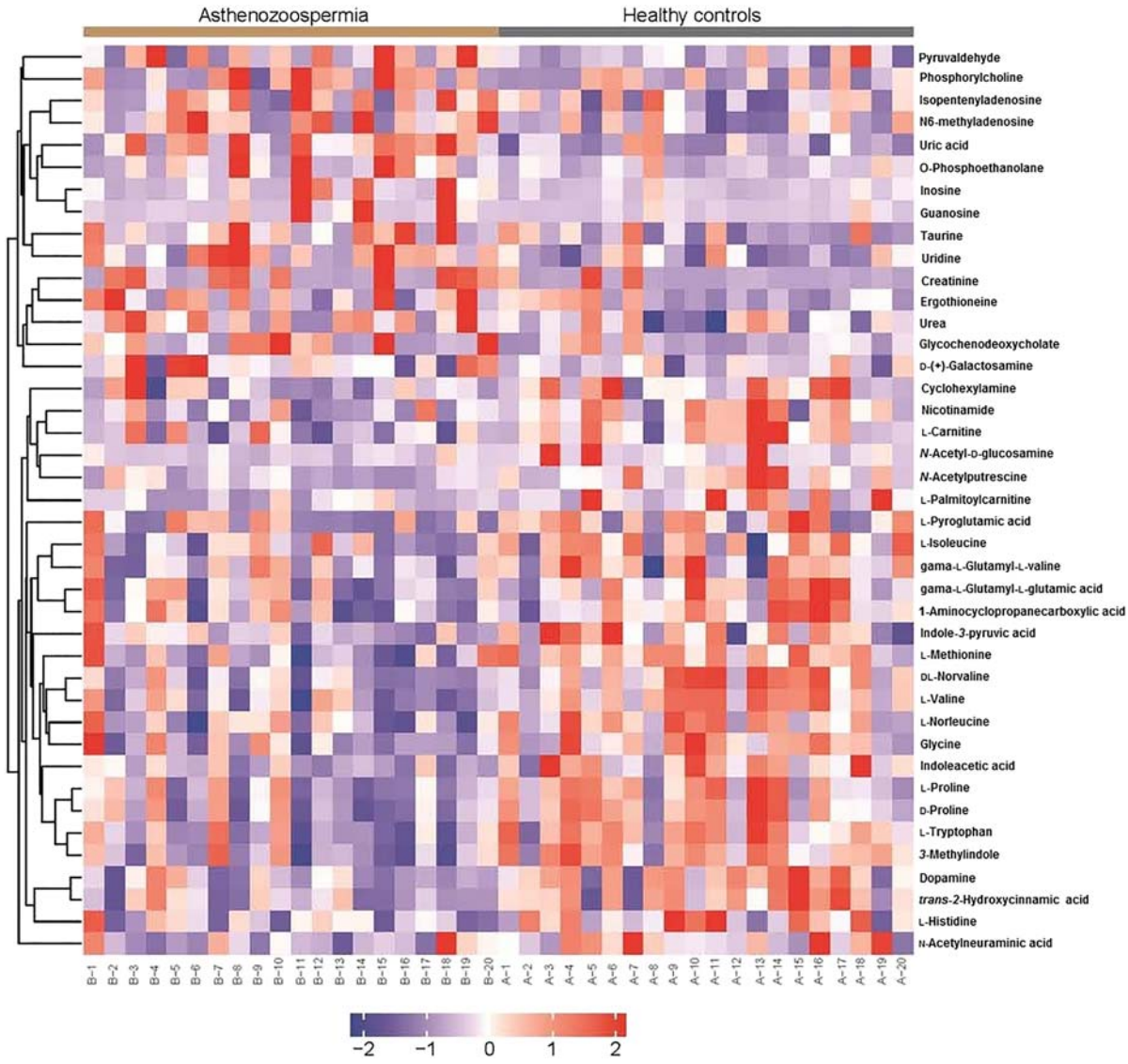

FIGURE 3 Hierarchical cluster analysis results of differential seminal plasma metabolites between asthenozoospermic patients and healthy controls. Red indicates upregulation and blueindicates downregulation. The columns and rows represent experimental seminal plasma samples and metabolites, respectively

TABLE 2 List of differential semen metabolites in asthenozoospermia patients compared with healthy controls

Metabolite	Rt (s)	*m/z*	VIP	Fold change	*p* value
Creatinine	197.973	114.067	5.475	2.532	.0217
Uric acid	297.185	169.037	2.95035	1.524	.000342
N^6-methyladenosine	129.963	282.123	8.432	1.4788	.01928
Uridine	245.039	245.0798	1.476	1.3673	.00304
Taurine	169.199	126.023	1.0436	1.3023	.0355
L-Carnitine	342.219	162.115	20.2161	0.7959	.0223
Nicotinamide	53.001	123.057	7.7887	0.72726	.0142
N-Acetylputrescine	363.369	131.119	1.42546	0.5798	.00999
L-Palmitoylcarnitine	153.821	400.344	2.288	0.5536	.01331

Note: Fold change value refers to the 'asthenozoospermia versus control group' change values.
Abbreviations: *m/z*, mass-to-charge ratio; Rt, retention time; VIP, variance importance for projection.

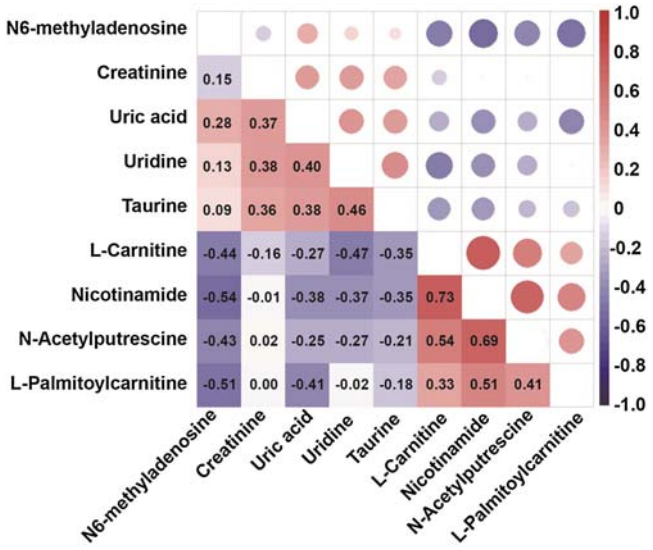

FIGURE 4 Heat map visualization of correlation analysis of differential metabolites. The colored dots indicate that the correlations between seminal plasma metabolites have statistical significance ($p < .05$). The red and blue dots represent positive and negative correlations, respectively

Metabolic regulation and energy support are essential for the normal process of spermatogenesis, yet this homeostasis may be disrupted in AS patients (Rato et al., 2012). In our study, elevated levels of taurine, uridine, uric acid and creatinine, which are related to essential amino acid metabolism, energy metabolism and nucleotide metabolism, were observed in patients with AS and not in healthy controls.

The levels of taurine, creatinine, uric acid and uridine in the semen of patients with AS were significantly increased, similar to another report using proton NMR spectroscopy or HPLC (Lazzarino et al., 2018; Zhang et al., 2015). Taurine is partly produced by oxidative metabolism of cysteine and is partly dependent on dietary intake from animal and fish protein (Stipanuk & Ueki, 2011). In the current study, cysteine was not significantly elevated. Therefore, it seems reasonable to assume that the increase in taurine in patients with AS was due to a high taurine diet. Concomitantly, elevated creatinine levels were observed in the seminal plasma of patients with AS, which has also been shown in a serum study of an infertile population and implies that energy or choline metabolism was probably disturbed (von Versen-Hoynck et al., 2019). The role of uric acid in the formation of spermatozoa remains unclear. Some studies have investigated the level of uric acid in different groups, and found that the concentrations of uric acid for normal men are higher than for those infertile men (Srivastava, Chopra, & Dasgupta, 1984; Xu et al., 2004), implying its positive effect on sperm function. Uric acid is also one of the major antioxidants in human semen that can counteract the damaging effect of oxidizing (e.g. reactive oxygen species and toxins) and nitrating agents (Andersen, 2004; Banihani, 2018). However, there are also other reports which indicate that higher levels of urate could attenuate endothelial nitric oxide synthase activity or inhibit the activity of creatine kinase and thus decrease the production of nitric oxide or adenosine triphosphate, which subsequently causes reduction in sperm motility (Banihani, 2018; Banihani, Abu-Alhayjaa, Amarin, & Alzoubi, 2018; Durany, Carreras, Valenti, Camara, & Carreras, 2002; Miraglia et al., 2011; Park et al., 2013). Therefore, it is reasonable to suggest that high levels of uric acid in human semen, similar to our current result, may reduce sperm motility and function, but further additional studies are needed to confirm this finding.

One interesting and unique finding from our study was that seminal plasma m^6A was significantly increased in patients with AS compared with healthy controls. This is the first study to establish and detect the increase in seminal plasma m^6A by metabolomic methods, with results implying a role for RNA epigenetic alterations in human AS. m^6A, which is an endogenous urinary nucleoside product of the degradation of transfer RNA, is important in RNA stability, splicing regulation, microRNA attenuation, RNA editing prevention, disease acceleration and epigenome control (Saletore, Chen-Kiang, & Mason, 2013). Although m^6A modification was first reported in early 1974 (Desrosiers, Friderici, & Rottman, 1974), its biological function and significance in fertilization remain largely unknown. Recently, it was reported that sperm m^6A is significantly increased and negatively correlated with sperm motility in human AS, which was proven to be

TABLE 3 Correlation of differentially significant metabolites and clinical sperm kinetics

	Sperm concentration	Sperm motility	VCL	VSL	VAP	ALH
Creatinine	0.085	−0.384*	−00.273	−0.132	−0.276	−0.256
Uric acid	−0.396*	−0.465**	−0.563*	−0.457**	−0.516**	−0.532**
N^6-methyladenosine	−0.470**	−0.382*	−0.377*	−0.310	−0.367*	−0.382*
Uridine	−0.213	−0.424**	−0.388*	−0.420**	−0.409**	−0.427**
Taurine	−0.261	−0.338*	−0.406**	−0.323*	−0.413**	−0.398*
L-Carnitine	0.574**	0.408**	0.254	0.330*	0.290	0.284
Nicotinamide	0.591**	0.366*	0.377*	0.400*	0.391*	0.403**
N-Acetylputrescine	0.277	0.394*	0.398*	0.279	0.385*	0.385*
L-Palmitoylcarnitine	0.520**	0.401*	0.592**	0.350*	0.514**	0.475**

Abbreviations: ALH, amplitude of lateral head displacement; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.
*$p < .05$.
**$p < .01$.

associated with the expression of one of the demethylases, alpha-ketoglutarate- and Fe^{2+}-dependent dioxygenase fat mass and obesity-associated protein (FTO; Ding et al., 2018). Thus, the current results strongly implicate that the seminal plasma level of m^6A is a potential biomarker of human infertility with AS.

In addition to the aforesaid elevated metabolites, in this study, the levels of L-carnitine, L-palmitoylcarnitine, nicotinamide and *N*-acetylputrescine were significantly downregulated in patients with AS, compared with healthy controls. L-Carnitine has been widely used as a valuable supplementation in the clinic, either alone or in combination with other (Mongioi et al., 2016) medications, to treat AS.

The level of nicotinamide was first reported to be significantly decreased in asthenozoospermic patients, compared with healthy controls, indicating a decreased level of NAD^+. Nicotinamide is an active form of vitamin B_3 and functions as a component of the coenzyme nicotinamide adenine dinucleotide (NAD), which also inhibits poly(ADP-ribose) polymerases, enzymes involved in the rejoining of DNA strand breaks induced by radiation or chemotherapy. In another recent report, the nicotinamide adenine dinucleotide reduced (NADH) dehydrogenase 1 alpha subcomplex (NDU FA13) was shown to be significantly downregulated in patients with AS, with lower mitochondrial membrane potential, higher intracellular reactive oxygen species levels and more apoptotic cells (Yang et al., 2017). Because most animals cannot manufacture this compound in amounts sufficient to prevent deficiency, it would be worth proving whether the intake of supplements, especially the current widely used alternative form of vitamin B_3, nicotinamide riboside, could benefit asthenozoospermic patients. However, the regulation of energy production involves numerous metabolic pathways, and its biological interpretation remains complex, so further investigation is needed.

4.1 | Limitations

There are some limitations in this study. First, the sample size is limited. Second, further validation on a larger population was not performed. Finally, the metabolomic profiles present only the downstream alterations of this disease, and thus, it is better to continue to study integrated genomics and proteomics in order to obtain a comprehensive understanding of etiology and the molecular mechanism of male infertility, especially about these significant metabolites and their exact functions.

5 | CONCLUSIONS

In this study, using an improved, more sensitive approach of the UHPLC–Q-TOF/MS technology, we investigated the difference in seminal metabolomics patterns between asthenozoospermic patients and healthy controls. Unique seminal metabolomic features were found in AS patients compared with controls. Our findings suggest that nicotinamide analogs may serve as supplemental therapy for clinical AS and identify m^6A as an epigenetic modification, which implies the pathogenesis of male infertility. Further studies with larger samples are needed to prove the potential roles of these metabolites in the pathophysiology of AS.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

DATA AVAILABILITY STATEMENTS

The ion peaks of original metabolites in positive mode (Excel) used to support the findings of this study are available from the corresponding author upon request.

ORCID

Pei Fan https://orcid.org/0000-0002-6592-3071

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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