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A COMPREHENSIVE REVIEW OF METABOLOMICS IN MALE INFERTILITY

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Abstract

Male infertility is a prevalent concern affecting nearly half of infertile couples, often shrouded in diagnostic complexity. Metabolomics, an emerging field, holds great promise for revolutionizing the diagnosis of male infertility by examining the full spectrum of small molecule metabolites within biological samples. The review showcases how metabolomics has identified potential biomarkers in male infertility, unveiling metabolic disruptions associated with conditions like azoospermia, oligoasthenoteratozospermia, asthenozoospermia, and oligozoospermia. It distinguishes targeted and untargeted metabolomics, elucidating their roles in investigating specific pathways or conducting holistic metabolite profiling. The authors highlight the fact that bridging the gap between metabolomics and reproductive medicine is crucial to fully exploit its diagnostic and therapeutic possibilities.

A. INTRODUCTION

The word metabolomics is derived from “Metabol” for metabolites and “Omics” meaning large scale analysis. Other disciplines in biology with suffix omics include genomics, transcriptomics, pharmacogenics, epigenomics, proteomics etc. [1,2, 3]

Metabolomics is the large-scale study of small molecules, commonly known as metabolites, within cells, biofluids, tissues or organisms. The interactions of these small molecules within a biological system are known as the metabolome. The study range includes:

a) Their presence in cells or organs

b) The ways they are altered in disease states

c) Their changes over time as a result of environmental stimuli

Metabolomics is a promising new field that has been suggested as a potential solution for diagnosing male infertility. While diagnostic semen analysis may fail to identify 50% of male infertility disorders, metabolomics may be able to provide more comprehensive information about metabolic processes in the body that could be contributing to infertility. [4, 5] The study of metabolomics in infertile men is in the first steps of research. Few studies in which human seminal plasma, urine or plasma have been used as biological sources for finding the potential biomarkers of infertile men are available.

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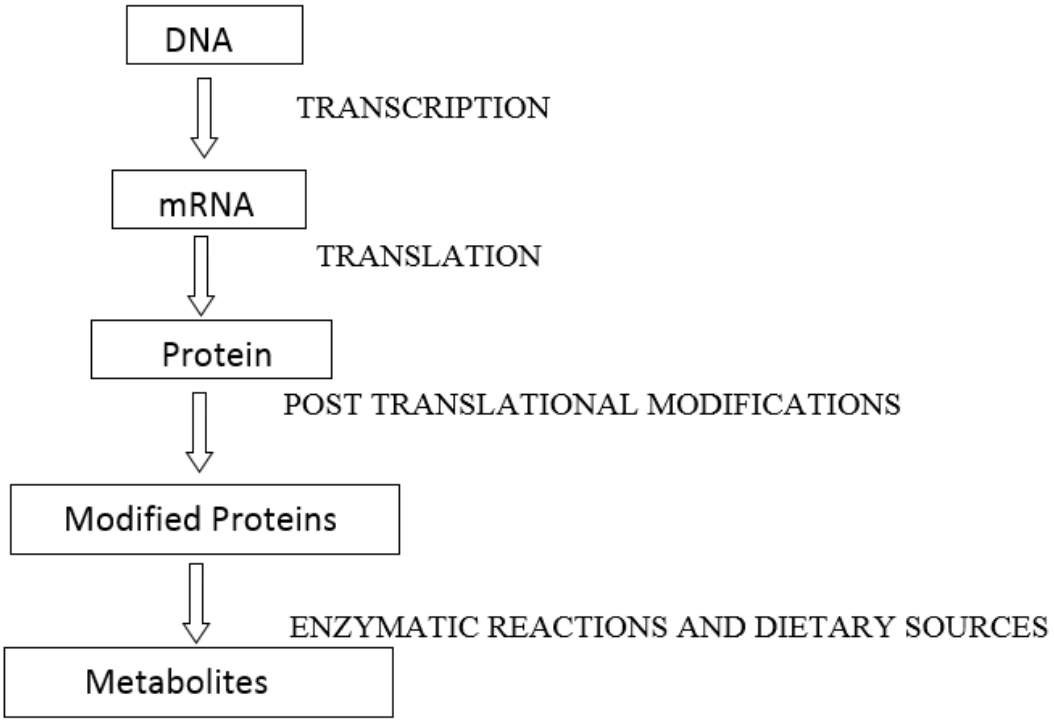

The genome is the complete set of genetic instructions encoded in an organism's DNA. It contains all the information necessary for the development and function of an organism. [6]

The transcriptome is the set of all RNA molecules transcribed from the genome of a cell or tissue. This includes messenger RNA (mRNA), which codes for proteins, as well as non-coding RNAs, which have various regulatory functions. [7,8]

The proteome is the complete set of proteins expressed by a cell, tissue, or organism. Proteins are the functional workhorses of cells, and they carry out many of the processes necessary for life.

The metabolome is the complete set of small molecule metabolites (e.g., sugars, amino acids, lipids) present in a cell, tissue, or organism. Metabolites are the end products of cellular processes and are often used as indicators of cellular activity.

Integrating these different datasets can provide a comprehensive understanding of the pathophysiology of renal disease. For example, genomic and transcriptomic data can be used to identify genetic mutations and changes in gene expression that contribute to the development of renal disease. Proteomic data can reveal changes in protein expression or post-translational modifications that may be involved in disease progression. Metabolomic data can provide insights into changes in metabolic pathways and help identify biomarkers of disease. By integrating these datasets, researchers can gain a more complete understanding of the complex molecular mechanisms underlying renal disease and develop more effective treatments. [9]

B. BRIEF HISTORICAL NOTE

Metabolites have been important in our understanding of life, health, and disease for thousands of years. The profiling of human disease using blood and urine samples has been carried out for centuries. Metabolomics began with the detection of high sugar content in urine by traditional Chinese in 1500-2000 BC. A urine wheel was published in 1506 by Ullrich Pinder, in his book Epiphanie Medicorum. The wheel described the possible colors, smells and tastes of urine, and used them to diagnose the diseases. Advances in analytics, pattern recognition, and metabolic profiling were made between 1940-1970, with mass spectrometry coupled with chromatography in the 1950s allowing for the development of new profiling technologies. [10]

Targeted metabolomics: Targeted metabolomics is a powerful analytical approach that focuses on the quantification and analysis of a predetermined set of metabolites, often referred to as a panel or pathway. This method allows researchers to investigate specific biochemical pathways or metabolic processes by measuring the concentrations of known metabolites involved in those pathways.

Targeted metabolomics is commonly used in various fields, including pharmacokinetic studies of drug metabolism. By analyzing specific metabolites related to drug metabolism, researchers can gain insights into how drugs are processed and metabolized in the body. This information is valuable for understanding drug efficacy, safety, and potential interactions with other substances.

Additionally, targeted metabolomics is employed to assess the impact of therapeutics or genetic modifications on specific enzymes. By quantifying metabolites associated with a particular enzyme's activity, researchers can evaluate the enzyme's function and measure the effects of drugs, genetic mutations, or other interventions on its activity.

Compared to untargeted metabolomics, which aims to profile a broad range of metabolites in a sample, targeted metabolomics provides a more focused and quantitative analysis of specific metabolites of interest. This targeted approach allows for higher sensitivity, selectivity, and reproducibility, making it particularly useful for addressing well-defined research questions in various areas of biomedical research, including drug development, personalized medicine, and biomarker discovery. [11]

Untargeted metabolomics: Unlike targeted metabolomics, which focuses on a predefined set of metabolites, untargeted metabolomics aims to comprehensively analyze and characterize all metabolites present in a biological sample without prior knowledge or bias towards specific compounds. [12]

Untargeted metabolomics involves the use of advanced analytical techniques such as mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy to generate a global metabolic profile. These techniques generate large datasets containing information on the masses, fragmentation patterns, and intensities of detected metabolites in the sample.

The main advantage of untargeted metabolomics is its ability to reveal new or unexpected metabolites that may not have been previously identified or characterized. This approach allows researchers to gain a comprehensive understanding of the metabolic landscape and discover novel metabolic pathways, biomarkers, or metabolic alterations associated with various biological processes, diseases, or environmental factors

By comparing metabolic profiles between different sample groups (e.g., healthy vs. diseased, treated vs. untreated), untargeted metabolomics can detect changes in metabolic pathways, identify potential biomarkers of diseases or drug responses, and provide insights into the underlying molecular mechanisms involved.

However, untargeted metabolomics also presents challenges in terms of data processing, metabolite identification, and data interpretation due to the large number of detected features and the complexity of metabolite annotation. Advanced computational and statistical methods, as well as databases of metabolite information, are used to analyze and annotate the data generated from untargeted metabolomics experiments.

Overall, untargeted metabolomics is a powerful tool for discovering new metabolites, understanding global metabolic changes, and generating hypotheses for further investigation in various fields, including biology, medicine, and environmental sciences. [13, 14]

Sample Preparation

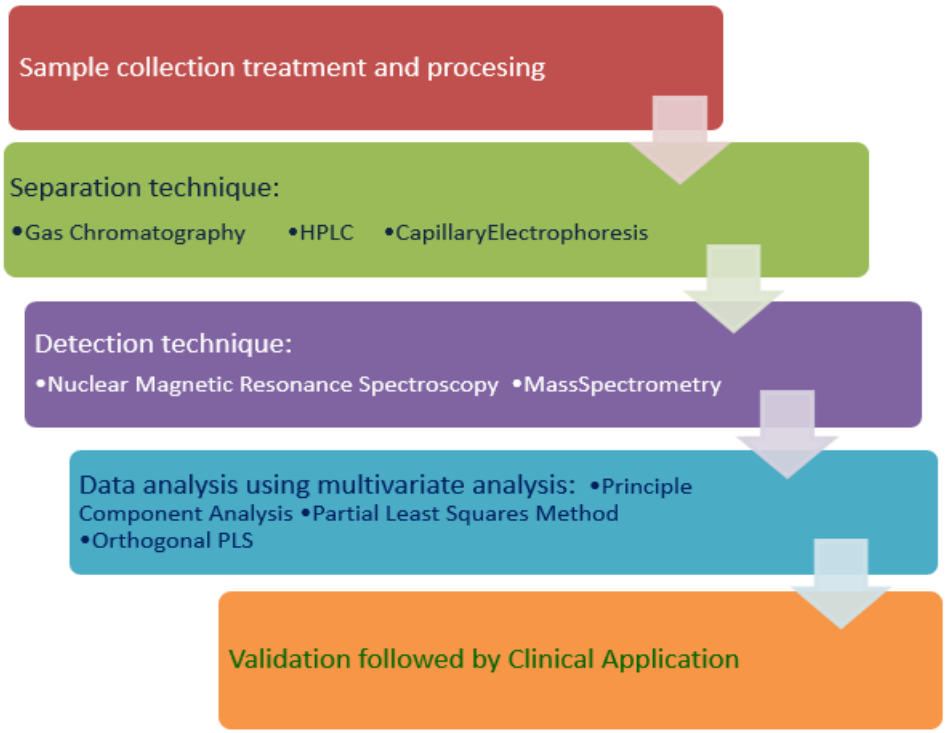

Figure No 1: Basic Workflow

Extraction of metabolites is typically done using aqueous or methanolic solvents, and the resulting extract is then fractionated into lipophilic (fat-soluble) and polar (water-soluble) phases. These phases are analyzed separately to capture different classes of metabolites. Further fractionation within each phase may occur to separate metabolites into specific classes before analysis.

Metabolomics serves as a bridge between an organism's genotype (genetic information) and phenotype (observable traits). By studying metabolites, researchers can gain insights into how genes are expressed and how they contribute to the overall characteristics of an organism.

Analyzing metabolomic data sets and their metadata, along with data mining techniques, can lead to the discovery of new hypotheses and potential targets for biotechnology applications.

Metabolomic biomarkers and male infertility: An Important Factor

There is a male factor involved in up to half of all infertile couples. Potential etiologies in male factor infertility are many and require thorough evaluation for their accurate identification. A complete medical history in conjunction with a focused examination can allow for an appropriate choice of laboratory and imaging studies. The semen analysis is a crucial first step, but by no means is it sufficient to determine a specific etiology or dictate therapy.

Molecular markers in male infertility are specific molecules, such as proteins, nucleic acids, or metabolites, that exhibit altered expression, function, or presence in individuals with infertility. These markers provide valuable insights into the underlying molecular mechanisms and can be used for diagnostic, prognostic, or therapeutic purposes. Some examples of molecular markers associated with male infertility:

I. Sperm-specific proteins: Proteins that are predominantly expressed in spermatozoa, such as SPINK2, TEX101, and ADAM3, have been identified as potential biomarkers for male infertility. Aberrant expression or absence of these proteins can indicate sperm dysfunction or impaired fertilization ability. [15]

II. Oxidative stress markers: Increased levels of reactive oxygen species (ROS) and oxidative stress have been linked to male infertility. Molecular markers of oxidative stress, including lipid peroxidation products (malondialdehyde, 4-hydroxynonenal) and antioxidant enzymes (superoxide dismutase, glutathione peroxidase), can indicate sperm damage caused by oxidative stress. [16, 17]

III. DNA damage markers: DNA fragmentation and chromosomal abnormalities in sperm are associated with infertility. Molecular markers such as phosphorylated histones (γH2AX) or DNA fragmentation assays (TUNEL assay, Comet assay) can assess DNA integrity and help identify individuals with compromised sperm DNA. [18, 19]

IV. Hormonal markers: Hormonal imbalances can contribute to male infertility. Measuring levels of reproductive hormones, such as testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and inhibin B, can provide insights into the hormonal status and identify potential causes of infertility. [20]

V. Genetic markers: Genetic abnormalities, such as microdeletions of the Y chromosome (AZF region) or mutations in genes related to sperm function (e.g., CFTR gene in cystic fibrosis), can lead to male infertility. Genetic testing or screening for specific gene mutations can serve as molecular markers for identifying genetic causes of infertility. [21]

The current review focuses on the application of metabolomics in the study of male infertility. The authors conducted a comprehensive search using various databases such as PubMed, Scopus, and Google Scholar, using keywords related to metabolomics and male infertility, such as "metabolomics," "metabolomics," "metabolomic profiling," "metabolomics fingerprinting," and "metabolome."

The human seminal plasma proteome holds untapped potential for identifying biomarkers linked to male infertility and reproductive disorders. By analyzing the proteins in seminal fluid using advanced techniques like mass spectrometry, researchers can detect dysregulated proteins associated with sperm function, motility, fertilization, and immune response modulation. These biomarkers have the potential to improve infertility diagnosis, personalize treatment strategies, predict the success of assisted reproductive techniques, and guide the development of targeted therapies. Exploring the seminal plasma proteome offers an exciting opportunity to transform our understanding of male infertility and provide hope for couples struggling with reproductive difficulties. Human seminal plasma has long been recognized as an important source for studying male infertility. One of the early studies mentioned in the review was conducted by Hamamah et al., where they utilized NMR technology to analyze human seminal plasma samples. They examined four different groups: spermatogenic failure subjects, vasectomized individuals, very severe oligoasthenozoospermia (OAT) subjects, and normozoospermia subjects (control group).

The studies by Hamamah et al. [22], Muncu et al.,[23] Zhang et al., [24] and Murgia et al. [25]

Investigated the metabolic alterations associated with male infertility using metabolomics approaches. These studies aimed to identify potential biomarkers and gain insights into the underlying mechanisms of impaired sperm function and production.

Proteomics Studies in Male Infertility

Azoospermia, characterized by the complete absence of sperm in semen, poses a significant challenge to male fertility. Hamamah et al. [22] conducted a study that focused on azoospermic individuals and explored the proteomic changes associated with this condition. They found that several proteins exhibited significant alterations in the azoospermic group compared to the control group. These proteins were involved in various biological processes, including spermatogenesis, sperm motility, and cellular metabolism. One of the key proteins identified in this study was glyceryl phosphorylcholine, which showed significant changes in levels. This finding suggests that glyceryl phosphorylcholine may play a role in the pathogenesis of azoospermia. Additionally, citrate and lactate, two metabolites crucial for energy production and cellular metabolism, also exhibited significant alterations in the azoospermic group. Furthermore, the ratios of citrate to lactate and glyceryl phosphorylcholine to lactate differed significantly between the control group and individuals with spermatogenic failure or those who had undergone vasectomy. These changes in metabolite ratios may provide valuable insights into the underlying metabolic disruptions in azoospermia.

Oligoasthenoteratozospermia is a condition characterized by reduced sperm count, motility, and abnormal sperm morphology. Muncu et al. [23] conducted a proteomics study to investigate the protein expression profiles in individuals with this condition. Their findings revealed significant alterations in the levels of various proteins related to sperm function and development. One of the notable observations was the lower levels of lactate, citrate, and several amino acids, including lysine, arginine, valine, and glutamine, in the oligoasthenoteratozospermia group compared to the normozoospermia control group. These changes indicated disruptions in energy

metabolism and amino acid utilization, which are crucial for sperm motility and overall functionality. Moreover, creatinine, α-ketoglutaric acid, spermine, and putrescine, all of which play roles in cellular processes related to sperm function, were found to be decreased in the oligoasthenoteratozospermia group. These findings suggest that alterations in protein expression related to energy metabolism, antioxidant defense, and cellular processes may contribute to the development of oligoasthenoteratozospermia.

Asthenozoospermia, characterized by reduced sperm motility, is another common male infertility-related condition. Zhang et al. [24] focused on this condition and conducted a proteomics study to explore the protein expression changes associated with asthenozoospermia. Their findings revealed significant alterations in several proteins involved in various cellular processes.

This study identified decreased levels of tyrosine and phenylalanine, two essential amino acids, in the asthenozoospermia group compared to the control group. Amino acids are critical for energy metabolism and protein synthesis, and their decreased levels may contribute to impaired sperm motility in asthenozoospermia. Additionally, increased levels of cholesterol, citrate, α-ketoglutaric acid, creatinine, choline, phosphocholine, glycerophosphocholine, cysteine, glutamine, and histidine were observed in the asthenozoospermia group. These changes indicated metabolic alterations related to amino acid metabolism, energy metabolism, and cellular processes in individuals with asthenozoospermia.

Seminal plasma plays a vital role in supporting sperm function and survival in the female reproductive tract. Murgia et al. [25] conducted a proteomics study to analyze the protein composition of human seminal plasma and identified several proteins with altered levels in individuals with male infertility-related conditions.

One of the significant findings was the decreased levels of fructose, myo-inositol, aspartate, and choline in the seminal plasma of individuals with male infertility. These alterations suggested potential disruptions in energy metabolism, cell signaling, protein synthesis, and cell membrane integrity within the seminal plasma.

Oligozoospermia, characterized by a reduced sperm count, is a common male infertility condition. Zhang et al. [26] took a metabolomics approach by analyzing urine samples to identify metabolite differences associated with the risk of oligozoospermia. Their study revealed significant changes in metabolite profiles that provided insights into the metabolic disruptions associated with this condition. In individuals at risk of oligozoospermia, decreased levels of acylcarnitine, aspartic acid, and leucylproline were observed. These alterations indicated potential disruptions in energy metabolism and protein synthesis, which are essential for sperm production and function. Moreover, increased levels of adenine and methylxanthine were found in the same group, suggesting altered purine metabolism and potential increased caffeine intake.

Mehrparavar et al. [4] emphasized the potential of metabolomics as a powerful tool for understanding the metabolic processes involved in sperm production and function. Their study identified alterations in metabolites related to energy metabolism, oxidative stress, and sperm maturation processes. The findings from this study highlighted the potential of metabolomics to improve diagnostic accuracy by identifying specific metabolic signatures associated with male infertility. By unraveling the metabolic pathways and dysregulations in individuals with male infertility-related conditions,

metabolomics offers valuable insights that can inform personalized treatment strategies.

Proteomics and Metabolomics: A Promising Approach in Andrology

Proteomics and metabolomics have emerged as promising tools for investigating the molecular mechanisms underlying male infertility. These advanced techniques enable researchers to explore the intricate web of proteins and metabolites that regulate the physiological functions of spermatozoa. While traditional semen analysis and clinical assessments remain valuable, proteomics and metabolomics offer a more comprehensive and detailed view of the molecular landscape associated with male infertility.

One of the significant challenges in the field of male infertility is the high prevalence of idiopathic cases where the cause of infertility remains unknown. Proteomics and metabolomics provide an opportunity to bridge this knowledge gap by identifying specific molecular biomarkers, metabolic signatures, and protein expression patterns associated with various infertility-related conditions. These insights may ultimately aid in the diagnosis and management of male infertility.

The studies reviewed in this article collectively demonstrate the potential of proteomics and metabolomics to uncover the molecular underpinnings of male infertility-related conditions such as azoospermia, oligoasthenoteratozospermia, asthenozoospermia, and oligozoospermia. The identification of specific proteins, metabolites, and metabolic pathways associated with these conditions offers valuable insights into their pathogenesis.

CONCLUSION

Metabolomics holds immense promise in reshaping our understanding of male infertility and advancing diagnostic capabilities. While it parallels clinical chemistry with its comprehensive and cost-effective approach, its unique advantages lie in simultaneously analyzing a multitude of metabolites. Beyond their conventional roles in metabolism, metabolites are emerging as crucial signaling molecules in the intricate web of male reproductive health. However, it's crucial to acknowledge that despite its potential, the application of metabolomics in male infertility is still in its infancy. The scarcity of experts trained in both metabolomics and reproductive medicine has hindered its clinical integration. Addressing this gap and fostering collaboration between these disciplines will be pivotal in harnessing the full potential of metabolomics to uncover biomarkers and etiological factors in male infertility, ultimately improving diagnosis and treatment outcomes.

Conflict of Interest: All Authors have no Conflicts of Interest.

References

1) Karahalil B. Overview of Systems Biology and Omics Technologies. Curr Med Chem. 2016 Sep 26; 23. doi: 10.2174/0929867323666160926150617.

2) Aderem A. Systems Biology: Its Practice and Challenges, Cell 2005;12: 511–13.

3) Kovac JR, Pastuszak AW, Lamb DJ. The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. Fertil Steril. 2013; 99(4):998–1007.

4) Mehrparavar B, Minai-Tehrani A, Arjmand B, Gilany K. Metabolomics of Male Infertility: A New Tool for Diagnostic Tests. J Reprod Infertil. 2019 Apr-Jun; 20(2):64-69.

5) Lombo, M.; Ruiz-Díaz, S.; Gutiérrez-Adán, A.; Sanchez-Calabuig, M.-J. Sperm Metabolomics through Nuclear Magnetic Resonance Spectroscopy. Animals 2021, 11, 1669. https://doi.org/10.3390/ani11061669.

6) Brown TA. Genomes. 2nd edition. Oxford: Wiley-Liss; 2002. Chapter 3, Transcriptomes and Proteomes. Available from: https://www.ncbi.nlm.nih.gov/books/NBK21121/

7) Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine; Micheel CM, Nass SJ, Omenn GS, editors. Evolution of Translational Omics: Lessons Learned and the Path Forward. Washington (DC): National Academies Press (US); 2012 Mar 23. 2, Omics-Based Clinical Discovery: Science, Technology, and Applications. Available from: https://www.ncbi.nlm.nih.gov/books/NBK202165/.

8) Qiu S, Cai Y, Yao H, Lin C, Xie Y, Tang S, Zhang A. Small molecule metabolites: discovery of biomarkers and therapeutic targets. Signal Transduct Target Ther. 2023 Mar 20; 8:132. doi: 10.1038/s41392-023-00888-2.

9) Tounta V, Liu Y, Cheyne A, Larrouy-Maumus G. Metabolomics in infectious diseases and drug discovery. Mol Omics. 2021 Jun 14; 17(3):376-393. doi: 10.1039/d1mo00017a. PMID: 34125125; PMCID: PMC8202295.

10) Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, Knox C, Bjorndahl TC, Krishnamurthy R, Saleem F, Liu P, Dame ZT, Poelzer J, Huynh J, Yallou FS, Psychogios N, Dong E, Bogumil R, Roehring C, Wishart DS. The human urine metabolome. PLoS One. 2013 Sep 4; 8(9):e73076. doi: 10.1371/journal.pone.0073076.

11) Roberts LD, Souza AL, Gerszten RE, Clish CB. Targeted metabolomics. Curr Protoc Mol Biol. 2012 Apr;Chapter 30:Unit 30.2.1-24. doi: 10.1002/0471142727.mb3002s98. PMID: 22470063; PMCID: PMC3334318.

12) Fu J, Zhu F, Xu CJ, Li Y. Metabolomics meets systems immunology. EMBO Rep. 2023 Apr 5; 24(4):e55747. doi: 10.15252/embr.202255747. Epub 2023 Mar 14. PMID: 36916532; PMCID: PMC10074123.

13) Gilany K, Minai-Tehrani A, Savadi-Shiraz E, Rezadoost H, Lakpour N. Exploring the human seminal plasma proteome: an unexplored gold mine of biomarker for male infertility and male reproduction disorder. J Reprod Infertil. 2015 Apr-Jun; 16(2):61-71.

14) Ma P, Zhang Z, Zhou X, Luo J, Lu H, Wang Y. Characterizing semen abnormality male infertility using non-targeted blood plasma metabolomics. PLoS One. 2019 Jul 5; 14(7):e0219179.

15) Suri A. Sperm specific proteins-potential candidate molecules for fertility control. Reprod Biol Endocrinol. 2004 Mar 10; 2:10.

16) Alahmar AT. Role of Oxidative Stress in Male Infertility: An Updated Review. J Hum Reprod Sci. 2019 Jan-Mar; 12(1):4-18.

17) Mannucci A, Argento FR, Fini E, Coccia ME, Taddei N, Becatti M, Fiorillo C. The Impact of Oxidative Stress in Male Infertility. Front Mol Biosci. 2022 Jan 5; 8:799294.

18) Christopher L.R. Barratt and others, Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications—a position report, Human Reproduction, Volume 25, Issue 4, April 2010, Pages 824–838.

19) Pizzol, Damiano, Bertoldo, Alessandro and Foresta, Carlo. "Male infertility: biomolecular aspects" Biomolecular Concepts, vol. 5, no. 6, 2014, pp. 449-456.

20) Oduwole OO, Huhtaniemi IT, Misrahi M. The Roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in Spermatogenesis and Folliculogenesis Revisited. Int J Mol Sci. 2021 Nov 25; 22(23):12735.

21) Kamiński P, Baszyński J, Jerzak I, Kavanagh BP, Nowacka-Chiari E, Polanin M, Szymański M, Woźniak A, Kozera W. External and Genetic Conditions Determining Male Infertility. International Journal of Molecular Sciences. 2020; 21(15):5274.

22) Hamamah S, Seguin F, Barthelemy C, Akoka S, Le Pape A, Lansac J, et al. 1H nuclear magnetic resonance studies of seminal plasma from fertile and infertile men. J Reprod Fertil. 1993; 97(1):51–5.

23) Muncu A, Karaer A, Dogan B, Tuncay G. Metabolomics analysis of seminal plasma in patients with idiopathic Oligoasthenoteratozoospermia using high-resolution NMR spectroscopy

24) Zhang X, Diao R, Zhu X, Li Z & Cai Z. (2015) Metabolic characterization of asthenozoospermia using nontargeted seminal plasma metabolomics. Clin Chim Acta 450, 254–261.

25) Murgia F, Corda V, Serrenti M, Usai V, Santoru ML, Hurt KJ, Passaretti M, Monni MC, Atzori L, Monni G. Seminal Fluid Metabolomic Markers of Oligozoospermic Infertility in Humans. Metabolites. 2020 Feb 11; 10(2):64.

26) Zhang J, Huang Z, Chen M, Xia Y, Martin FL, Hang W, Shen H. Urinary metabolome identifies signatures of oligozoospermic infertile men. Fertil Steril. 2014 Jul; 102(1):44-53. e12.