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A Systematic Review of Novel Tools and Techniques for Understanding Molecular Mechanisms in Sperm Metabolism and Development

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Abstract: Male infertility is a multifactorial condition often associated with disruptions in sperm metabolism and mitochondrial function, yet traditional semen analysis provides limited insight into these molecular mechanisms. Understanding sperm bioenergetics and metabolic dysfunctions is crucial for improving diagnosis and treatment of conditions such as asthenozoospermia and azoospermia. **Methods:** This systematic review synthesizes recent literature, focusing on novel tools and techniques—including omics technologies, advanced imaging, spectroscopy, and functional assays—that enable comprehensive molecular assessment of sperm metabolism and development. **Results:** The reviewed studies highlight the effectiveness of metabolomics, proteomics, and transcriptomics in identifying metabolic biomarkers linked to male infertility. Non-invasive imaging modalities such as Raman and magnetic resonance spectroscopy offer real-time metabolic profiling, while the seminal microbiome is increasingly recognized for its role in modulating sperm metabolic health. Despite these advances, challenges remain in clinical validation and implementation of these techniques in routine infertility diagnostics. **Conclusions:** Integrating novel molecular metabolic assessments with conventional semen analysis promises enhanced diagnostic precision and personalized therapeutic approaches, ultimately improving reproductive outcomes. Continued research is needed to standardize biomarkers and validate clinical utility. Furthermore, these metabolic tools hold significant potential to elucidate the underlying causes of previously misunderstood and unexplained infertility cases, offering new avenues for diagnosis and treatment.

Keywords: sperm metabolism; mitochondrial dysfunction; male infertility; metabolomics; proteomics; molecular biomarkers; seminal microbiome; advanced imaging; sperm bioenergetics

1. Introduction

Human spermatozoa are highly specialized cells responsible for delivering the paternal genome to the oocyte, a process that requires substantial energy primarily in the form of adenosine triphosphate (ATP) to maintain sperm integrity and motility [1]. Motility alone consumes the majority of sperm ATP, which is generated mainly through glycolysis and oxidative phosphorylation

(OXPHOS) pathways localized in distinct sperm compartments [2,3]. These metabolic pathways are dynamically regulated to meet the energy demands during sperm development, maturation, capacitation, and transit through the male and female reproductive tracts [4]. Despite its critical role, sperm metabolism and bioenergetics remain insufficiently explored, limiting advances in diagnosing and treating male infertility conditions such as asthenozoospermia and azoospermia [5].

Infertility affects approximately 8–12% of couples worldwide, with male factors contributing to nearly half of these cases [6,7]. Among male infertility disorders, sperm motility defects have been increasingly linked to mitochondrial dysfunction and impaired energy metabolism [8]. Emerging evidence also implicates the seminal and sperm-associated microbiome in modulating sperm DNA integrity and metabolism, suggesting novel therapeutic targets [9]. The advent of omics technologies—including genomics, proteomics, and metabolomics—combined with advanced analytical tools, now enables comprehensive profiling of sperm metabolic pathways and identification of molecular biomarkers associated with fertility outcomes [10–12].

This systematic review synthesizes recent advances in novel tools and techniques for evaluating human sperm metabolism and development. It emphasizes methods that uncover molecular mechanisms underlying infertility and improve prediction of sperm retrieval success, particularly in azoospermia and metabolic impairments. Highlighting the central role of mitochondria and oxidative stress in idiopathic infertility, this work aims to guide future research and clinical strategies to enhance male fertility diagnostics and therapeutics.

2. Methods

2.1. Sources

Data were independently extracted by the authors. A systematic search was conducted through PubMed, Scopus, and Web of Science databases, covering records published from each database inception to 15 February 2025. The search strategy combined Medical Subject Headings (MeSH) terms and keywords tailored to capture relevant literature on sperm metabolism and development.

Specifically, key terms included: “sperm metabolism”, “sperm bioenergetics”, “glycolysis”, “oxidative phosphorylation”, “mitochondrial function”, “mitochondrial membrane potential”, “sperm motility”, “asthenozoospermia”, “azoospermia”, “male infertility”, “metabolomics”, “proteomics”, “transcriptomics””, antioxidants”, “sperm capacitation”, and “microbiome”.

Additional manual searches were performed by screening the reference lists of pertinent articles to identify further relevant studies not captured by database queries.

2.2. Study Selection

Studies that met the following inclusion criteria were included in the qualitative synthesis to focus on evaluating novel tools and techniques for assessing sperm metabolism and development in relation to male infertility:

1. Design: Original research articles including observational studies, clinical trials, case reports, and technical method evaluations; reviews and small cohort studies were excluded.
2. Patient inclusion criteria: Human males with infertility of various etiologies, including idiopathic infertility and metabolic-related dysfunctions affecting sperm quality.
3. Patient exclusion criteria: Review articles, studies involving animal models; male infertility with well-established causes such as varicocele, cryptorchidism, infections of male accessory glands, genetic abnormalities (e.g., Y chromosome microdeletions), testicular torsion or trauma, and systemic diseases including thyroid, pituitary, adrenal disorders, kidney or liver failure.
4. Study intervention: Application or evaluation of diagnostic or research tools and techniques aimed at identifying biomarkers or characterizing sperm metabolic pathways and developmental processes.

5. Study outcome: Assessment of the utility, accuracy, and relevance of advanced methods and emerging technologies for evaluating sperm metabolism and their usefulness in diagnosing or understanding metabolic sperm-related infertility.

3. Results

Using the described search strategy, 2,775 records were initially identified. After removing duplicates, 2,433 records remained. Screening titles and abstracts led to the exclusion of 977 records as not pertinent, leaving 1,456 articles for further assessment. Of these, 1,150 papers were excluded with reasons during detailed screening. A total of 306 full-text articles were assessed for eligibility, with 263 excluded for not satisfying the inclusion criteria. Finally, 43 studies met the criteria and were included in this systematic review (Figure 1).

This review synthesizes the most relevant and recent tools and techniques used to evaluate human sperm metabolism and development, focusing on their application in male infertility diagnostics and research. The included studies provide a comprehensive overview of methodologies assessing metabolic pathways, mitochondrial function, and molecular biomarkers associated with various infertility causes.

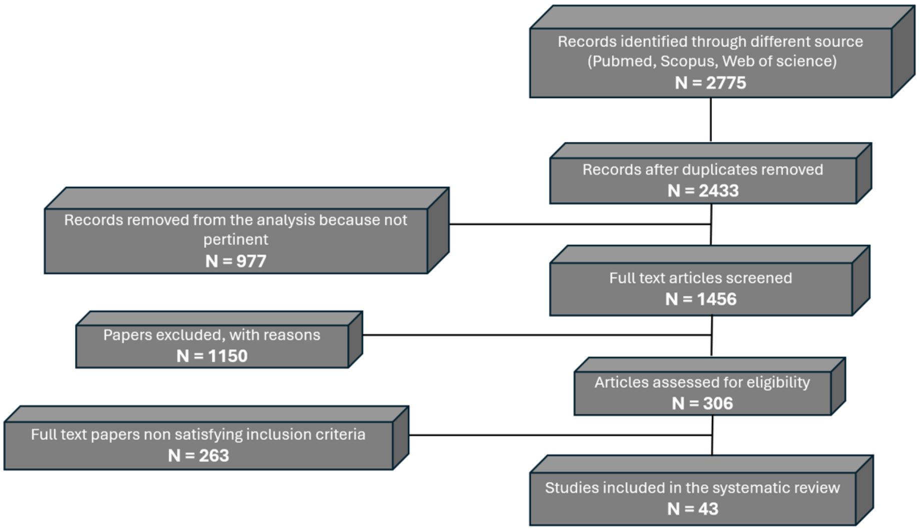

Figure 1. Flowchart detailing the selection of studies for inclusion in the review. PRISM.

Before discussing the findings on these novel techniques and their clinical relevance, subsequent sections summarize current knowledge on sperm metabolism and its critical role in male fertility, providing the foundation for future research and clinical applications.

4. Energy Metabolism Pathways in Spermatozoa

Energy metabolism in spermatozoa depends on a series of tightly regulated biochemical reactions culminating in the production of adenosine triphosphate (ATP), the cell's primary energy currency [13]. Two principal pathways generate ATP: glycolysis and oxidative phosphorylation (OXPHOS). Glycolysis occurs mainly in the sperm's principal piece and head, providing a rapid but limited ATP supply without requiring oxygen [2] . This pathway yields only two net ATP molecules per glucose molecule.

OXPHOS, in contrast, takes place in the mitochondria densely packed within the sperm midpiece. This oxygen-dependent pathway is more efficient and produces the bulk of ATP needed to meet the high energy demands of sperm motility [3]. ATP generated in the midpiece must be transported along the flagellum to power movement. While diffusion has been proposed as a mechanism, its efficiency is limited by the narrow space within the flagellum, suggesting specialized transport systems might be involved, though these remain to be fully elucidated in humans and other mammals [14,15].

5. Energy Metabolism Pathways in Spermatozoa

5.1. Metabolic Substrates and Transport

Sperm utilize both endogenous and exogenous carbohydrates to satisfy their metabolic needs. Seminal plasma from various mammals, including humans, contains fructose as the main metabolic substrate [16,17], along with glucose and, in some species, mannose [18]. These sugars are internalized through glucose transporters (GLUTs) with varying substrate specificities [19,20]. Additionally, other sugars such as sorbitol serve as energy substrates in species including humans, rams, and mice [21,22].

Mitochondrial substrates include lactate and pyruvate, metabolized via lactate dehydrogenase (LDH) to regenerate NAD+ or enter the Krebs cycle for OXPHOS [23]. Pyruvate and lactate may originate from glycolysis or be taken up externally via monocarboxylate transporters [24,25]. Human sperm express specific LDH isoforms that enhance mitochondrial utilization of lactate, permitting efficient respiratory substrate use [26,27]. Fatty acid β-oxidation also contributes to ATP production. Boar and bull sperm metabolize long-chain fatty acids such as oleic, palmitic, and myristic acids via mitochondrial β-oxidation [28,29]. Human sperm possess enzymatic machinery for both saturated and unsaturated fatty acid β-oxidation; inhibition of this pathway impairs motility [30]. Glycogen reserves, once thought absent in mature sperm, have been found in some species such as dogs, where sperm can synthesize and utilize glycogen as an energy source [15,31,32].

5.2. Metabolic Adaptations During Maturation and Transit

Many studies use epididymal sperm to assess basal metabolic activity; however, epididymal sperm lack exposure to seminal plasma, which provides a fluid rich in energetic substrates and metabolic modulators [33]. Differences in metabolic activity between epididymal and ejaculated sperm have been observed. For example, ejaculated bull sperm rely heavily on OXPHOS for motility, whereas epididymal sperm maintain ATP levels primarily through glycolysis [33–35]. In mice, epididymal sperm depend mainly on glucose, but ejaculated sperm utilize a broader range of substrates, including citrate and pyruvate, through nonglycolytic pathways [36].

Sperm acquire fertilizing capacity through capacitation, a process involving extensive biochemical and physiological changes such as plasma membrane remodeling, ion fluxes, and activation of signaling cascades including cAMP-dependent pathways. Metabolic activity increases substantially during capacitation, reflecting heightened energy demands [37]. During transit through the female reproductive tract, sperm encounter variable environments with differing oxygen and substrate availability, necessitating metabolic adaptability to maintain motility and viability until fertilization [36,38,39].

6. Molecular Biomarkers and Oxidative Stress

Disruptions in sperm metabolism are frequently characterized by mitochondrial dysfunction and heightened oxidative stress, both of which play pivotal roles in the pathophysiology of male infertility. Mitochondria, being the powerhouse of sperm cells, are essential for ATP production necessary for motility and overall sperm function. However, excessive generation of reactive oxygen species (ROS) — a byproduct of impaired mitochondrial electron transport chains — leads to oxidative damage targeting mitochondrial membranes, proteins, and mitochondrial DNA [40]. This oxidative insult not only diminishes sperm motility but also compromises the integrity of nuclear DNA, increasing the risk of mutations and fragmentation that can adversely affect fertilization and embryo development [4].

Advances in high-throughput omics technologies, including proteomics, metabolomics, and transcriptomics, have enabled the identification of specific molecular biomarkers that serve as indicators of these metabolic disturbances. Proteomic analyses have revealed aberrant expression of key mitochondrial proteins and antioxidant enzymes, while metabolomic profiling has uncovered altered concentrations of metabolites involved in energy metabolism and redox balance. Transcriptomic studies further highlight dysregulation in gene expression pathways related to oxidative stress response and mitochondrial function [11]. Together, these molecular signatures provide valuable insight into the biochemical underpinnings of sperm dysfunction and hold promise for developing diagnostic tools and targeted therapies aimed at mitigating oxidative damage in infertile men.

7. Influence of the Seminal Microbiome

Recent research has begun to elucidate the significant role of the seminal microbiome—the diverse microbial communities residing within seminal fluid and associated with spermatozoa—in modulating sperm metabolic health and fertility outcomes. These microbial populations can influence the biochemical milieu of the seminal plasma, impacting factors such as pH, nutrient availability, and oxidative stress levels. Certain bacterial species have been implicated in either protective or detrimental effects on sperm function, with some microbes producing metabolites that exacerbate oxidative stress or trigger inflammatory responses, thereby compromising sperm DNA integrity and metabolic efficiency [9]. The interaction between the seminal microbiome and sperm cells represents a complex and dynamic system that may contribute to idiopathic cases of male infertility. Understanding how microbial dysbiosis alters sperm metabolism and function opens new avenues for therapeutic intervention, such as the use of probiotics, antibiotics, or microbiome modulation strategies to restore a healthy seminal environment. This emerging field holds promise for enhancing fertility treatments by addressing not only the host's cellular dysfunction but also the microbial factors influencing reproductive health.

8. Advanced Techniques and Tools for Evaluating Sperm Metabolism

The evaluation of sperm metabolism and its role in male infertility has advanced considerably through diverse methodologies encompassing proteomics, metabolomics, transcriptomics, imaging, and functional assays. Agarwal et al. conducted proteomic analyses on seminal plasma from 42 infertile men and 17 fertile donors, identifying a 35-protein pathway linked to sperm dysfunction, including overexpression of membrane metallo-endopeptidase (MME), which was significantly elevated in infertile patients [41]. Similarly, Amaral et al. characterized the sperm tail proteome in normozoospermic men, identifying 1,049 proteins with a prominent presence of lipid metabolism enzymes and active mitochondrial and peroxisomal pathways, highlighting the metabolic complexity essential for sperm motility [8].

Metabolomic studies have revealed significant biochemical alterations in infertile populations, Boguenet et al. applied targeted metabolomics to seminal plasma from severe oligoasthenospermia patients, documenting decreased metabolites associated with sperm membrane deterioration and energy defects [42]. Complementing this, Li et al. identified nine metabolites differing significantly in asthenozoospermic men, with nicotinamide levels correlating positively with sperm count and motility [43]. Zhao et al. detected 33 disturbed metabolites in idiopathic asthenozoospermia, implicating disrupted energy and amino acid metabolism [44], while in another study he reported 72 to 89 metabolites altered in oligospermic patients, including key amino acid and ketone body pathways [44]. Mitochondrial dysfunction emerges as a central theme linking these molecular changes to impaired sperm function. Cassina et al. demonstrated that mitochondrial respiratory deficits and nitro-oxidative protein modifications in sperm midpiece and head regions correlated with reduced motility in their cohort [40]. This functional impairment aligns with fluorescence-based assessments by Paoli et al. who showed a positive correlation between mitochondrial membrane

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potential—measured by JC-1 staining—and sperm motility, reinforcing mitochondrial health as a fertility marker [45].

Transcriptomic and epigenetic analyses have further enriched biomarker discovery. Larriba et al. profiled small RNAs in seminal extracellular vesicles from azoospermic men, identifying canonical and isomiR microRNAs that effectively discriminate azoospermia origin with high specificity [46]. Li et al. reported 238 differentially methylated regions in asthenozoospermic spermatozoa, affecting 114 genes essential for spermatogenesis and motility, suggesting epigenetic regulation as a contributor to metabolic dysfunction [43]. Imaging and spectroscopic techniques provide complementary functional insights. Raman microspectroscopy has been utilized to detect sperm DNA damage, enabling sensitive biochemical characterization suitable for live sperm in a study by Costa et al. [47]. Additionally, 13C-magnetic resonance spectroscopy revealed that motile sperm preferentially metabolize glucose, fructose, and pyruvate into lactate, highlighting increased glycolytic activity crucial for motility [48]. These observations align with metabolic profiling studies that differentiated metabolite peaks between sperm populations using 1H magnetic resonance spectroscopy [49].

Oxidative stress remains a critical factor in sperm dysfunction, with Aziz et al. and Irigoyen et al. quantifying reactive oxygen species (ROS) production in sperm cells [50,51]. Their studies demonstrated that increased hydrogen peroxide and peroxynitrite levels inversely correlate with mitochondrial respiratory control ratios and sperm motility, highlighting oxidative damage as a major disruptor of bioenergetics. Proteomic analyses have identified 15 proteins that are differentially expressed in spermatozoa exhibiting elevated reactive oxygen species, especially those related to energy metabolism and antioxidant defense [11]. Emerging research further implicates the seminal microbiome in sperm health; combining 16S rRNA sequencing with untargeted metabolomics revealed an enrichment of Lactobacillus iners in men with a high sperm DNA fragmentation index, associating microbial metabolites such as butanoate fermentation products with DNA damage and metabolic disruptions [9].

The integration of these multi-dimensional data sets has been facilitated by bioinformatics approaches. Milardi et al. and Sharma et al. utilized pathway enrichment and network modeling to identify disrupted metabolic nodes in infertile men, accelerating biomarker discovery and therapeutic targeting [10,11]. Such systems biology approaches are crucial for translating complex molecular insights into clinical practice.

Collectively, these studies underscore that sperm metabolism—and its perturbations involving mitochondrial dysfunction, oxidative stress, epigenetic alterations, and microbial influences—plays a pivotal role in male infertility. The high sensitivity and specificity of these advanced methodologies promise improved diagnostic precision and personalized treatment strategies. Table 1 summarizes the key methods and their contributions, illustrating the strides made toward comprehensive sperm metabolism assessment beyond traditional semen analysis.

Table 1. Studies on Innovative Techniques in Sperm Metabolism and Male Infertility.

Authors	Study Aim	Methods/Tools	Results
Agarwal et al., 2015 [41]	Identify seminal plasma proteins involved in ROS-mediated male infertility	• Chemiluminescence assay • Proteomic analysis via 1D gel electrophoresis • In-gel digestion • LC/MS-MS	• Membrane Metallo-Endopeptidase (MME) overexpressed; Proteins modulated in infertile groups • 35-protein pathway linked to sperm dysfunction
Almeida et al., 2011 [52]	Quantify testicular sperm apoptosis via active caspase-3 in normal and impaired spermatogenesis	• Fluorescence microscopy	• Higher active caspase-3 rates in obstructive azoospermia (OA) and hypospermatogenesis

			• Distinct localization patterns
Amaral et al., 2013 [8]	Characterize human sperm tail proteome, focusing on metabolism-related proteins	• LC-MS/MS • Sonication and sucrose-gradient ultracentrifugation for tail fractions	• Identified 1049 proteins identified; lipid metabolism enzymes prominent • Mitochondrial and peroxisomal pathways active
Aziz et al., 2010 [50]	Determine cell type contributions to intracellular H2O2 and peroxynitrite production in sperm	• Chemiluminescence • Flow cytometry with fluorescent probes	• Cell-type specific ROS production • H2O2 and peroxynitrite correlated
Barceló et al., 2018 [53]	Evaluate seminal plasma exosomal mirnas as markers for Azoospermia origin and sperm presence	• MiRNA quantitative PCR panels • Exosome isolation	• Mir-31-5p as biomarker for azoospermia origin combined with FSH improves prediction
Boguenet et al., 2020 [42]	Assess metabolomic signatures of seminal plasma in severe Oligoasthenospermia	• Targeted quantitative metabolomics • HPLC-TANDEM MS	• Decreased metabolites linked to sperm membrane deterioration and energy defects
Calle-Guisado et al., 2017 [54]	Identify and localize AMP-activated protein kinase (AMPK) in human sperm and evaluate its role in sperm motility	• Western blotting • Indirect immunofluorescence	• AMPK localized in acrosome, midpiece, tail • Active AMPK higher in high motility sperm; inhibition reduces motility
Calvert et al., 2019 [48]	Investigate sperm metabolism	• Magnetic Resonance Spectroscopy (MRS)	• Sperm metabolize glucose, fructose, pyruvate to lactate • Motile sperm show higher glycolytic activity
Cassina et al., 2015 [40]	Analyze mitochondrial function and oxidative stress in human sperm affecting fertility	• High-resolution respirometry • Oxidative/nitrative protein detection	• Mitochondrial dysfunction correlated with reduced motility with nitro-oxidative modifications in midpiece and head
Costa et al., 2018 [47]	Assessement of sperm DNA damage and biochemical features	• Raman microspectroscopy with principal component analysis	• DNA damage detected only in dry conditions • Technique applicable to live sperm analysis
Deng et al., 2024 [55]	Profile seminal plasma lipid composition in necrozoospermia and evaluate lipid biomarkers	• LC-MS lipidomic analysis	• Identified 1267 lipids; 20 lipids predictive for necrozoospermia • Lipidomics improves diagnosis
Fietz et al., 2024 [56]	Discover seminal plasma biomarkers for non-invasive differential diagnosis of Obstructive Azoospermia (OA) vs. Non-Obstructive Azoospermia (NOA)	• Label-free LC-MS/MS proteomic analysis • Western blot validation	• 42 proteins downregulated in NOA SCO patients • Testis-specific proteins as biomarkers

Fukuda et al., 2016 [57]	Assess impact of seminal clusterin level on spermatogenesis and sperm retrieval in infertile men	• ELISA • Immunohistochemistry	• Seminal clusterin lower in NOA and oligozoospermia • Correlation with testicular expression • FSH and clusterin predict retrieval
Hashemitabar et al., 2015 [58]	Identify novel biomarkers for asthenozoospermia via sperm tail proteomic analysis	• 2D GEL ELECTROPHORESIS • MALDI-TOF-TOF MS	• 14 proteins altered in asthenozoospermia including tubulin beta 2B, glutathione S-transferase Mu 3, clusterin
He et al., 2024 [9]	Investigate seminal microbiome and metabolome role in high sperm DNA fragmentation index (HDFI)	• 16S rRNA SEQUENCING • UNTARGETED METABOLOMICS	• Lactobacillus iners enriched in HDFI • Microbial profiles linked to sperm DNA fragmentation • Butanoate fermentation implicated
Irigoyen et al., 2022 [51]	Assess sperm mitochondrial metabolism and ROS production as tools to complement semen analysis	• High-resolution respirometry • H2O2 measurement; • Antioxidant capacity assays;	• Mitochondrial respiratory control ratio correlates with motility; H2O2 inversely correlated
Jayaraman et al., 2014 [59]	Analyze seminal plasma metabolic profiles in idiopathic/male factor infertility	• 1H NMR spectroscopy (700 mhz)	• Distinct metabolic profiles in idiopathic infertility : Altered lysine, arginine, tyrosine, citrate, fructose
Kyrgiafini et al., 2023 [60]	Identify lncRNA mutations and expression in teratozoospermia	• Whole-genome sequencing • RNA expression analysis	• 1166 unique mutations in Differentially Expressed lncRNAs • Variants affect structure/function and MiRNA interactions
Larriba et al., 2024 [46]	Small RNA profiling in seminal extracellular vesicles for azoospermia classification	• High-throughput small RNA profiling • RT-qPCR validation	• Canonical and isomiR microRNAs discriminate azoospermia origin • tRNA and piRNAs less discriminatory
Li et al., 2022 [61]	Investigate DNA methylation patterns in asthenozospermia	• Whole-genome bisulfite sequencing	• 238 differentially methylated regions annotated to 114 genes related to spermatogenesis and motility
Liang et al., 2021 [62]	Proteomic profiling of sperm in severe oligoasthenoteratozoospermia	• Isobaric tag labeling; LC-MS/MS • Immunofluorescence western blot	• 938 proteins differentially expressed • Metabolic pathways enriched • YBX1 upregulated • AK1 and ACO2 downregulated

Liu et al., 2014 [63]	Identify spermatogenesis in testicular tissue	• Raman spectroscopy • Immunohistochemistry • Metabolomic analysis	• Raman spectra distinguished NOA from OA with ~90% sensitivity and 86% specificity
Martins et al., 2020 [64]	Characterize seminal plasma proteome in primary and secondary infertility	• Proteomic shotgun analysis • Western blot	• Dysregulated proteins linked to secretion and immune response
Milardi et al., 2014 [10]	Identify seminal biomarkers for secondary male hypogonadism (HH)	• LTQ ORBITRAP mass spectrometry • Protein interaction network	• 33 proteins absent in hypogonadic patients • 14 recovered after testosterone therapy
Olesti et al., 2023 [65]	Correlate metabolomic profiles with semen quality in young men	• Untargted metabolomics	• Metabolites including acyl-carnitines and steroids distinguish low vs. high sperm count
Olesti et al., 2020 [66]	Develop steroidomics strategy for human seminal fluid	• LC-HRMS • Extraction optimization	• Detected 41 steroids including androgens; steroid profile stable
Paiva et al., 2015 [67]	Comprehensive metabolomic characterization of human sperm cell	• 1H NMR AND GC-MS • 2D NMR SPECTROSCOPY	• Identified 69 metabolites • Pathways linked to sperm physiology and dysfunction
Paoli et al., 2011 [45]	Correlate sperm mitochondrial integrity with motility	• JC-1 fluorometric staining	• Positive correlation between mitochondrial membrane potential and sperm motility
Qiao et al., 2017 [68]	Metabolic profiling of unexplained male infertility (UMI)	• GC-MS	• Identified 44 differential metabolites • Metabolic pathways altered
Reynolds et al., 2017 [49]	Examine sperm molecules	• 1H MRS scanning of sperm	• Density gradient concentration (DGC) with two washes minimized contamination • Metabolite peaks differed between sperm populations
Sharma et al., 2013 [11]	Identify seminal plasma proteins as biomarkers of sperm quality	• LC-MS/MS PROTEOMIC ANALYSIS	• 20 proteins differentially expressed • Biological regulation affected
Sharma et al., 2013 [12]	Proteomic profile changes in spermatozoa with elevated ROS	• LC-MS/MS	• 15 proteins differentially expressed • Energy metabolism and oxidative stress pathways affected
Soler et al., 2016 [69]	Fertility-predictive model profiles of spermatozoa	• MALDI-TOF MS • TOP-DOWN PROTEIN IDENTIFICATION	• High diagnostic accuracy • Proteins involved in energy metabolism and sperm structure

Sulc et al., 2024 [70]	Phospholipid expression in Sertoli cell-only syndrome (SCOS) testis	• MALDI imaging mass spectrometry	• Phospholipid levels correlated with spermatogenesis • Potential microTESE diagnostic tool
Massana et al., 2021 [71]	Proteomic analysis of sperm in fertilization failure after ICSI	• 2D-LC-MS/MS • Western blot • Flow cytometry • Fluorometric assays	• Altered mitochondrial and proteasomal proteins in fertilization failure
Nitao et al., 2021 [72]	Progesterone-induced Ca2+ oscillations in human sperm	• Fluorescence imaging; • whole-cell current clamp • Pharmacological inhibitors	• Ca2+ oscillations generated by CatSper channels • Membrane potential modulates oscillations
Vashisht et al., 2021 [73]	Evaluate seminal plasma biochemical and immunological markers in male infertility	• Assays for 21 analytes in seminal plasma • ROC analysis	• 15 markers significantly altered • Monocytes/lymphocytes in NOA • Platelets in asthenozoospermia
Wainstein et al., 2023 [74]	Study microRNA profiles in semen and testicular tissue of azoospermic men	• Small RNA deep sequencing • RT-qPCR validation	• Mir-202-3p reduced in azoospermic semen • mir-370-3p elevated in azoospermia without sperm in testis
Wang et al., 2011 [75]	Seminal plasma miRNAs in infertile men and diagnostic value	• SOLEXA sequencing • RT-qPCR	• Seven miRNAs altered • miRNAs better diagnostic markers than routine parameters
Wu et al., 2020 [76]	DNA methylation in testicular cells of azoospermia patients	• Genome-wide DNA methylation • Transcriptome profiling	• Disordered methylation in NOA • Altered reproductive gene expression
Xu et al., 2018 [77]	Ribonuclease (RNASET2) levels in sperm and relation to motility	• Protein quantification • Incubation experiments • Immunoprecipitation • Mass spectrometry	• RNASET2 elevated in asthenozoospermia • Inversely correlated with motility • Interacts with AKAP4
Zhao et al., 2018 [44]	Metabolic profiling of idiopathic asthenozoospermia sperm cells	• Untargeted GC-MS metabolomics	• 33 metabolites identified • Disturbed energy and amino acid metabolism
Zhao et al., 2024 [78]	Semen metabolic profiling in oligospermia patients	• HPLC-MS/MS metabolomics	• 72-89 metabolites as potential markers • Altered amino acid and ketone body metabolism

9. Clinical Implications: Metabolic Dysfunction in Male Infertility

The reviewed studies underscore the critical role of metabolic dysfunction in prevalent infertility conditions such as asthenozoospermia and azoospermia. Alterations in energy metabolism pathways, mitochondrial dysfunction, and oxidative stress are recurrent themes linked to impaired sperm motility and viability [40,41,58]. In asthenozoospermia, metabolomic and proteomic studies have identified disrupted glycolysis and fatty acid oxidation pathways, correlating with reduced motility [8,43]. Similarly, azoospermic patients exhibit distinct proteomic and microRNA profiles predictive of spermatogenic status and sperm retrieval success [53,56,62]. These biomarkers provide valuable prognostic information, guiding clinical decisions and personalized treatment strategies.

Emerging evidence also highlights the influence of the seminal microbiome on sperm metabolic health and DNA integrity, adding complexity to infertility diagnostics [9]. Despite advances, challenges remain in standardizing assays and integrating multi-omics data into routine clinical practice [65,71].

The integration of advanced metabolic assessment tools into clinical workflows promises improved diagnosis, prognosis, and management of male infertility.

Future research should focus on validating these biomarkers in larger cohorts and developing accessible platforms for widespread clinical application.

10. Conclusions

The relationship between sperm metabolism and male fertility is increasingly recognized, yet its clinical application remains limited. Traditional semen analysis often fails to explain idiopathic infertility, highlighting the need for metabolic profiling and molecular biomarkers to improve diagnostic accuracy. These advanced tools provide deeper insights into mitochondrial function, energy metabolism, and oxidative stress—key factors for sperm quality and fertility. Challenges remain in identifying which patients will benefit most, determining optimal testing timing, and selecting the most informative biomarkers. Additionally, randomized clinical trials are needed to validate these approaches, especially for idiopathic infertility cases. Future research should focus on clinical validation and exploring targeted therapies that enhance sperm function.

Integrating metabolic assessments with conventional analysis promises more personalized treatments and better outcomes. By advancing biomarker-driven diagnostics, we move closer to resolving male infertility’s complexities and improving reproductive success through precision medicine.

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