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REVIEW

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Seminal plasma exosomes and male infertility: current progress and future directions

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Abstract

Male infertility remains a major global health concern. Conventional semen analysis offers limited explanatory power, with most cases remaining idiopathic, underscoring the urgent need for novel biomarkers and mechanisms. Exosomes, 30–150 nm extracellular vesicles that transfer proteins, RNAs, and bioactive molecules, have emerged as pivotal regulators of male reproductive function. Epididymosomes promote sperm maturation and capacitation by delivering proteins and antioxidants. Prostasomes act post-ejaculation, supporting capacitation, the acrosome reaction, and immune protection within the female reproductive tract. Testicular exosomes regulate early spermatogenesis, particularly by modulating spermatogonial stem-cell proliferation and differentiation. Seminal-vesicle exosomes influence motility and fertilization, while bulbourethral exosomes may modulate seminal pH, ion balance, and viscosity. Dysregulation of these exosomal pathways has been linked to oligozoospermia, asthenozoospermia, and teratozoospermia. This review integrates mechanistic and translational evidence across seminal plasma exosome subtypes, emphasizes their reproductive functions, and highlights their diagnostic and therapeutic potential, offering a foundation for exosome-based precision medicine in male infertility.

Keywords

Seminal plasma exosomes; Epididymosomes; Prostasomes; Testicular exosomes; Male infertility; Sperm maturation; Sperm function; Intercellular communication; Biomarkers

1. Introduction

Clinical infertility is defined as the inability of a couple to achieve conception after 12 months of regular, unprotected intercourse. Male factors account for approximately 30–50% of infertility cases [1], and are often associated with a range of underlying etiologies [2, 3]. Despite the high prevalence of male infertility, clinical diagnosis continues to rely predominantly on conventional semen analysis, which evaluates sperm concentration, motility, and morphology. However, these standard parameters account for only about 30% of male infertility cases, leading to a substantial proportion being classified as idiopathic [4]. This diagnostic limitation highlights the urgent need to elucidate the molecular mechanisms contributing to male infertility and identify novel biomarkers and therapeutic targets that can improve diagnostic precision and treatment outcomes.

Recent advances in extracellular vesicle research have highlighted the essential roles of exosomes in male reproduction. Exosomes are membrane-bound vesicles measuring 30–150 nm in diameter, released through exocytosis [5]. They originate from endocytic processes, during which early endosomes mature into multivesicular bodies (MVBs). Within MVBs, intraluminal vesicles (ILVs) are generated via Endosomal Sorting Complex Required for Transport (ESCRT)-dependent or ESCRT-independent pathways that involve specific lipids and tetraspanins such as Cluster of Differentiation 63 (CD63), CD81, and CD9 [6, 7]. The fusion of MVBs with the plasma membrane leads to the release of ILVs as exosomes into extracellular fluids, including blood, urine, and semen [8].

The understanding of exosome function has evolved considerably over time. Initially identified in 1983 as cellular waste disposal vesicles [9], exosomes were subsequently found to possess immunomodulatory properties in 1996 [10] and were later shown in 2007 to mediate RNA transfer between cells [11], collectively establishing exosomes as key mediators of intercellular communication. Through direct fusion with target cells, exosomes deliver their molecular cargo, thereby modulating recipient cell function [12].

Effective male reproduction relies on tightly coordinated intercellular communication, in which exosomal biomolecules play essential roles by mediating interactions across diverse cell types [13]. Seminal plasma exosomes (SPE), secreted by the epididymis, prostate, testis, seminal vesicles, and bulbourethral glands [14], contain a wide array of proteins, RNAs, and other bioactive molecules that influence sperm function and viability. The major subtypes of SPE include epididymosomes, prostasomes [15, 16], and testicular exosomes [17],

while exosomes derived from the seminal vesicles and bulbourethral glands are still under investigation. SPE are involved in regulating intercellular communication and modulating sperm motility, morphology, capacitation, acrosome reaction, and fertilization, in addition to providing protective effects [18]. Although previous reviews have broadly addressed extracellular vesicles in reproduction or focused on proteomic aspects, they have largely overlooked the integrated functional landscape of SPE subtypes and their translational potential. The present review addresses this gap by providing a systematic integration of all major SPE subtypes, including epididymosomes, prostasomes, testicular exosomes, and emerging populations from the seminal vesicles and bulbourethral glands, and elucidating their mechanistic roles in fertility through intercellular communication networks. It also discusses findings on bibliometric analysis to highlight current research hotspots and future directions, while proposing SPE-based diagnostic panels and therapeutic strategies. In contrast to studies on individual subtypes, this comprehensive approach delineates the coordinated interactions among exosomal populations, thereby establishing a conceptual framework for clinical translation, as a deeper understanding of SPE biology can clarify the underlying mechanisms of male infertility and facilitate the development of innovative biomarkers and therapeutic strategies (Fig. 1).

Since the landmark reviews by Simon *et al*. [18] (2018) and Wang *et al*. [14] (2022), numerous studies have further elucidated exosome-mediated mechanisms, identified novel biomarkers, and explored preclinical therapeutic applications. Despite these advances, the findings remain fragmented due to methodological inconsistencies and persistent barriers to clinical translation. Thus, this review also aims to consolidate recent evidence, highlight emerging opportunities, and propose standardized approaches to facilitate the clinical application of SPE research in the context of male infertility.

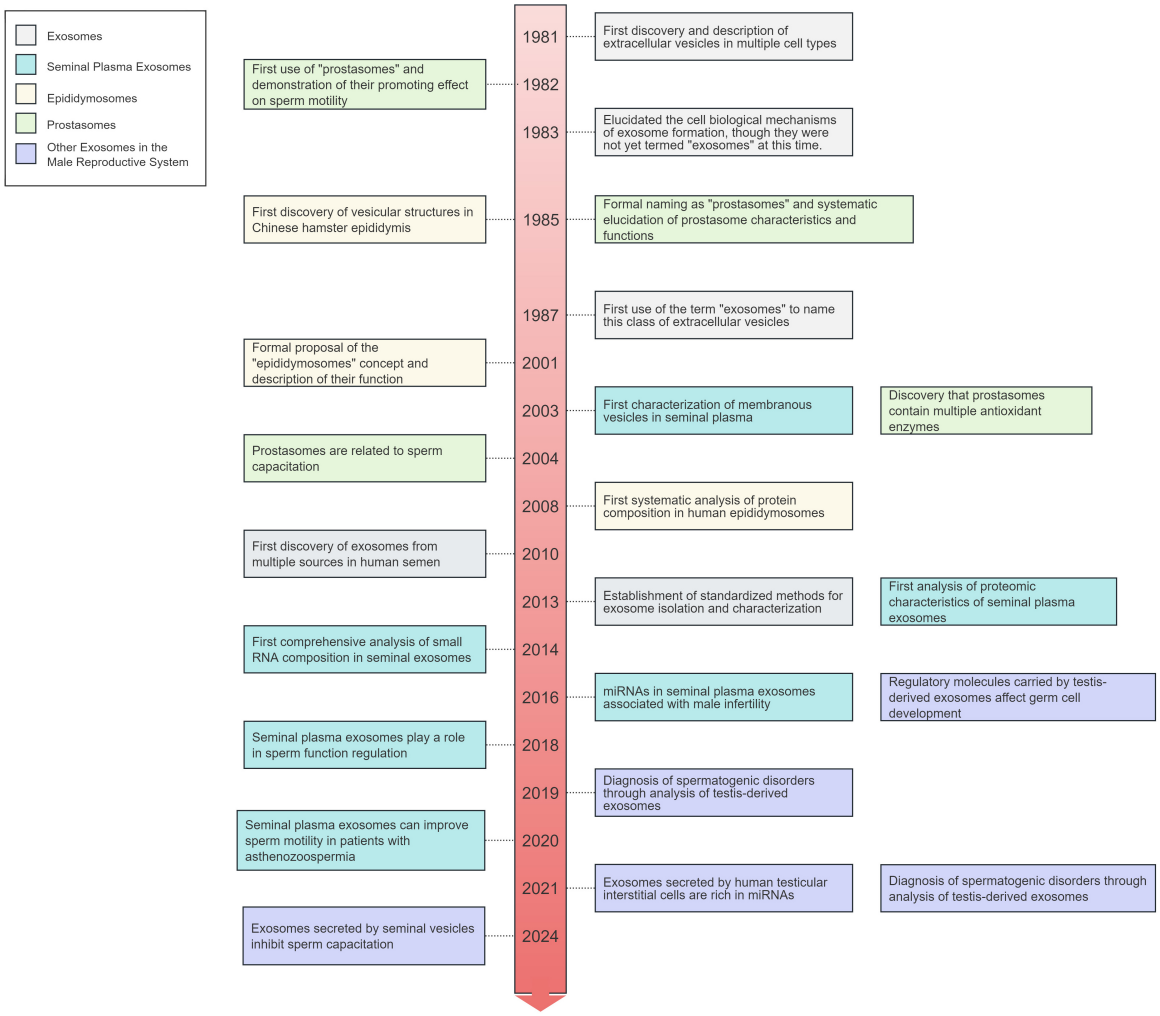

FIGURE 1. Key timeline of exosome research in the male reproductive system. Note: This timeline presents major milestones in exosome research related to the male reproductive system from 1981 to 2024. It highlights key discoveries in general exosome biology (grey), seminal plasma exosomes (light blue), epididymosomes (yellow), prostasomes (green), and exosomes derived from other components of the male reproductive tract (purple). The progression reflects the evolving understanding of how distinct exosome populations contribute to male fertility, beginning with their initial identification and extending to recent developments in clinical application.

2. Seminal plasma and exosomes

Research in the field of exosomes has progressed substantially over the past several decades. In 1981, Trams *et al*. [19] first described extracellular vesicles in various cell types, providing the foundation for the field. Subsequently, in 1983, Pan and Johnstone elucidated the mechanism of exosome formation through studies on transferrin receptor externalization during the maturation of sheep reticulocytes [9]. However, the term "exosome" was formally introduced by Johnstone *et al*. [20] in 1987.

Semen is formed by the dilution of concentrated epididymal sperm suspensions with secretions from accessory glands. Spermatozoa are produced in the testes, undergo maturation and acquire motility in the epididymis. During ejaculation, they are transported through the vas deferens, where they mix with prostatic and other glandular fluids. More than 95% of semen volume consists of seminal plasma, which is composed of secretions from the epididymis, prostate, testes, seminal vesicles, and bulbourethral glands. In 2009, Poliakov *et al*. [21] systematically identified exosomes in human seminal plasma, confirming their multi-source origins. Seminal plasma, traditionally considered a medium for sperm nutrition, transport, and protection, is now recognized to contain a wide array of proteins and bioactive molecules essential for sperm maturation and fertilization, as revealed by omics-based studies [22]. Characteristic seminal plasma proteins include Lipocalin-type Prostaglandin D Synthase (L-PGDS), Testis Expressed 101 (TEX101), and Extracellular Matrix Protein 1 (ECM1), which are used to assess reproductive tract patency in azoospermia. In this regard, Protein deglycase DJ-1 (DJ-1), is associated with reactive oxygen species (ROS)-related infertility [23]; and Transketolase-like 1 (TKTL1), Lactate Dehydrogenase C (LDHC), and Phosphoglycerate Kinase 2 (PGK2), serve as diagnostic markers of fertility potential [24]. Importantly, most seminal plasma proteins and bioactive compounds interact with sperm via exosome-mediated delivery, playing an important role in regulating sperm function [25].

Seminal plasma exosomes (SPE) can be reliably characterized using well-established methods. The standardization of exosome research has been advanced by the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines, which provide detailed criteria for exosome isolation and characterization [26, 27]. As with general exosome protocols, transmission electron microscopy (TEM) is used to visualize their characteristic cup-shaped bilayer structure [27–29], while nanoparticle tracking analysis (NTA) determines their size distribution, typically ranging from 30 to 150 nm [30, 31]. Western blotting is employed to detect positive exosomal markers, including transmembrane proteins such as CD63, CD9, and CD81, and cytosolic proteins such as Tumor Susceptibility Gene 101 (TSG101) and Alix [32–34]. Negative markers, such as calnexin, histones, and Golgi Matrix Protein 130 (GM130), are used to exclude contamination from non-exosomal sources [26, 35, 36]. According to MISEV guidelines, proper characterization requires detection of at least three positive markers (including both transmembrane and cytosolic types) and one negative marker [26, 37].

Despite methodological advances, the isolation of SPE remains challenging due to their relatively low concentrations and the presence of interfering proteins and other molecular components [31, 38]. Standard protocols typically involve sequential low-speed centrifugation to eliminate debris, followed by size exclusion chromatography (SEC), which enhances both purity and recovery [30, 36].

Proteomic analysis of SPE offers promising opportunities for biomarker discovery. However, SPE contains over 10,000 proteins [39, 40], and this high complexity, combined with substantial inter-individual variability [31, 39, 41], makes it difficult to detect proteins present at low abundance. To overcome this limitation, depletion strategies such as immunoprecipitation are used to selectively remove high-abundance proteins, allowing for improved detection of proteins potentially involved in male fertility. As proteomic techniques continue to advance, it is expected that more reliable diagnostic and therapeutic markers will be identified from SPE.

For seminal plasma, the isolation methods vary. For instance, although traditional ultracentrifugation remains in use, microfluidics and improved SEC have shown superior clinical performance [42]. Microfluidics simplifies the isolation process and addresses the limitations of conventional methods in terms of accuracy, efficiency, and practicality, thereby offering advantages for automation and point-of-care fertility assessment. Polymer precipitation provides high yields but poses a risk of contamination [43]. In contrast, SEC preserves exosome integrity and offers better purity, although with lower yields. It achieves over 95% albumin reduction while maintaining similar recovery of key markers [30, 44, 45]. The combination of ultracentrifugation and SEC improves both yield and purity, increases protein identification, and reduces contaminants [46] (Table 1, Ref. [44, 47–53]).

3. Epididymosomes

Epididymal cells exhibit high metabolic, endocytic, and secretory activity, and these processes are highly dependent on androgens, particularly dihydrotestosterone (DHT). After castration, the epididymal weight decreases to approximately 30% of the normal weight, emphasizing its androgen dependence [54]. The epithelium of the epididymis comprises six major cell types: principal cells (the predominant (~80%) type and responsible for protein absorption and secretion into the lumen), basal cells, clear cells, narrow cells, apical cells, and halo cells.

Spermatozoa, produced in the testis, enter the epididymis via the efferent ducts, primarily driven by luminal fluid flow and weak inherent motility. The epididymis facilitates sperm transport, concentration (via selective reabsorption of water and solutes to increase sperm density and optimize paracrine signaling), protection, storage, and the acquisition of motility and fertilization capacity, all of which are regulated by the luminal microenvironment. Oxidative stress contributes to the pathogenesis of male infertility [55], as the high metabolic activity of epididymal cells leads to ROS generation, which can impair sperm function. This oxidative burden can be partially mitigated by the secretion of antioxidant enzymes, such as superoxide dismutase [56].

Anatomically, the epididymis is divided into the caput (site

TABLE 1. Comparison of exosome isolation methods for seminal plasma.

Method	Principle	Advantages	Limitations	Processing Time	Sample Volume	Seminal Plasma Suitability
Ultracentrifugation (UC)	Density-based sedimentation at 100,000–120,000×g [47]	Gold standard [48]; high specificity for vesicle size [49]; well-established protocols [48]	Low efficiency; albumin contamination [44]; potential vesicle damage [47]; requires expensive equipment	3–4 h	1–5 mL	Good; viscosity may reduce efficiency
Size Exclusion Chromatography (SEC)	Size-based separation using porous beads	High purity [44]; preserves vesicle integrity; gentle isolation; suitable for downstream RNA analysis	Lower yield compared to precipitation; requires larger starting volume; limited concentration capability	30–60 min	0.5–2 mL	Excellent; suitable for viscous samples
Polymer Precipitation (PEG/ExoQuick)	Precipitation using polyethylene glycol [50]	High yield; simple protocol [51]; no specialized equipment; scalable	High protein contamination; co-precipitation of non-vesicular material; may alter vesicle properties; interferes with some downstream assays	12–16 h	0.2–1 mL	Moderate; protein interference is a concern
Immunoaffinity Capture	Antibody-based capture via surface markers (CD63, CD81, CD9)	High specificity for subpopulations; targets specific vesicle types; compatible with small volumes [52]	Expensive antibodies; may miss marker-negative vesicles; potential cross-reactivity; limited scalability	2–4 h	0.1–1 mL	Good for targeted subpopulation analysis
Microfluidics	Size or flow-based separation in microfabricated channels [44]	Rapid processing; small volume requirement; high-throughput potential; automation-compatible; suitable for point-of-care applications [44]	Requires specialized devices; risk of channel clogging; limited commercial availability; requires method optimization	10–30 min	0.05–0.2 mL	Excellent; well-suited for clinical applications
Combined UC + SEC	Sequential UC followed by SEC [53]	Higher purity than UC alone; higher yield than SEC alone; reduced contamination; improved protein identification [53]	Longer processing time; higher cost; more complex protocol; requires both instruments [53]	4–5 h	1–3 mL	Very good; balances yield and purity

PEG: Polyethylene Glycol; CD: Cluster of Differentiation.

of early sperm maturation), corpus (site of late maturation), and cauda (site of sperm storage), each characterized by a distinct luminal microenvironment [57]. Segment-specific differences in gene and protein expression, as well as in epididymosome distribution, have been observed [58], highlighting the need for further investigation into the region-specific functions, molecular compositions, and their respective roles in sperm maturation.

The discovery of epididymosomes has advanced the understanding of epididymal function. In 1985, membrane-bound vesicles interacting with sperm were observed in the epididymal fluid of the Chinese hamster [59]. In 2001, Frenette and Sullivan introduced the term "epididymosomes" and described their role in transferring proteins to sperm [60]. These vesicles are mainly produced and released by principal cells, which have high secretory and endocytic activity [61].

Epididymosomes, exosomes present in the epididymal lumen and derived from epithelial cells, nourish and protect transiting sperm together with other secretions [62, 63]. They are secreted via an apocrine mechanism, in which apical blebs protrude, detach, dissolve, and release their cargo [64]. During epididymal transit, they mediate communication with sperma-

tozoa and surrounding cells by transferring their contents [65] and they also contribute to capacitation [66].

The molecular composition of epididymosomes was first clarified in 2008 through a proteomic analysis by Thimon *et al*. [67], who examined human epididymosomes from vasectomy reversal fluid and identified hundreds of proteins. They contain diverse proteins, including miRNAs, mRNAs, and lipids involved in protein transport, oxidation-reduction, and metabolism [68]. Key molecules transferred during sperm maturation include P34H [69], Sperm Adhesion Molecule 1 (SPAM1) [70], Plasma Membrane Calcium-Transporting ATPase 4 (PMCA4) [16], Solute Carrier Family 27 Member 2 (SLC27A2), Epididymal Protein 3B (EDDM3B), Keratin 19 (KRT19), and WAP Four-Disulfide Core Domain Protein 8 (WFDC8) [65]. Additionally, epididymosomal proteins include Glutathione Peroxidase 5 (GPX5) [71], Biliverdin Reductase A (BLVRA) [72], and Epididymal Sperm-Binding Protein 1 (ELSPBP1) [72, 73], while A Disintegrin and Metalloproteinase Domain-Containing Protein 7 (ADAM7) plays a role in regulating sperm morphology and motility [74, 75].

4. Prostasomes

Prostasomes are exosomes secreted by prostatic epithelial cells into the prostatic acinar ducts and constitute a major component of seminal plasma [15, 76]. These vesicles, ranging from 30 to 200 nm in diameter, mix with spermatozoa and other glandular secretions during ejaculation, where they play key roles in sperm function and fertilization. The discovery of prostasomes began in 1977, when Ronquist and Hedström identified membrane-bound structures with Adenosine Triphosphatase (ATPase) activity in human prostatic fluid [77]. In 1982, Stegmayr and Ronquist demonstrated their ability to enhance sperm motility [78], and in 1985, Ronquist and Brody formally introduced the term “prostasomes” while characterizing their secretion and function [79]. In 2014, Vojtech *et al*. [80] identified a diverse RNA repertoire within prostasomes, suggesting their regulatory roles in sperm–egg interactions.

Freshly ejaculated spermatozoa are not fully capacitated. The role of prostasomes in regulating capacitation was clarified when Arienti *et al*. [81] showed that prostasome-mediated modulation of sperm function is pH-dependent, emphasizing the importance of the microenvironment in prostasome–sperm interactions. Prostasomes can interact and fuse with the sperm membrane, transferring their contents from prostatic secretory cells to spermatozoa, thereby promoting capacitation [82]. They also deliver cyclic adenosine monophosphate (cAMP), which activates protein kinase A and facilitates the capacitation process [83]. In addition, prostasomes can induce the acrosome reaction by fusing with spermatozoa and transferring signaling molecules such as cAMP and Ca^{2+} to regulate this process [84]. The female reproductive tract contains abundant natural killer (NK) cells, which form a natural immunological barrier during fertilization. Research has shown that prostasomes help protect spermatozoa within this environment. They contain immune-related proteins, such as CD48, which modulate the local immune environment, thereby shielding sperm from immune attack and increasing their survival rates [85]. Additionally, prostasomes also enhance sperm responsiveness to progesterone near the oocyte, a process closely linked to fertilization [86]. Overall, prostasomes are involved in multiple aspects of sperm function, including motility, semen liquefaction, immunosuppression, antioxidant activity, antimicrobial effects, acrosome reaction, and capacitation, which are essential for successful fertilization within the female reproductive tract.

5. Other exosomes in the male reproductive system

SPE from the testis, seminal vesicles, and bulbourethral glands complement the functions of epididymosomes and prostasomes, contributing to sperm function, reproductive health, and the pathogenesis of male infertility. These exosomes participate in complex intercellular communication networks that regulate spermatogenesis, sperm maturation, and fertilization, and they provide novel insights into the molecular mechanisms underlying male infertility.

The testis, as the primary site of spermatogenesis, contains seminiferous tubules composed of germ cells and Sertoli cells, along with interstitial cells such as Leydig cells and macrophages. These testicular cells secrete exosomes that coordinate testicular development and spermatogenesis through highly regulated communication pathways [87].

Spermatogonial stem cells (SSCs), which form the foundation of spermatogenesis, directly influence testicular development and determine sperm production capacity. Studies in multiple mammalian species have shown that spermatogonia secrete abundant exosomes at the basement membrane of seminiferous tubules [87]. These cell-specific exosomes are selectively taken up by SSCs and play a regulatory role in promoting their proliferation [88]. Notably, spermatogonial exosomes and their associated protein cargo have demonstrated significant diagnostic and therapeutic potential for male infertility. Exosome-based biological interventions targeting SSCs have shown promise across various experimental platforms [89].

Exosomes, as mediators of intercellular communication, exhibit multiple functions, including the promotion of spermatogenesis and improvement of sperm quality. In particular, they hold potential in the treatment of azoospermia, especially the non-obstructive azoospermia (NOA) subtype. Studies have indicated that exosomes derived from mesenchymal stem cells (MSCs) promote spermatogenesis through several mechanisms, such as anti-apoptotic effects, stimulation of testosterone secretion, and reduction of oxidative stress [90]. Although numerous preclinical studies support the safety and efficacy of exosomes and demonstrate improvements in sperm production in infertile animal models, no clinical trials have been completed to date, indicating that this field remains in the early stages of clinical translation. Among their potential therapeutic applications, the antioxidant properties of exosomes are particularly important, as they may help reduce sperm damage induced by oxidative stress [91]. However, despite these encouraging preclinical findings, the specific mechanisms of action, optimal dosage, treatment timing, and overall clinical safety of exosome-based therapies require further investigation and validation.

Sertoli cell-derived exosomes play key regulatory roles in

spermatogenesis, particularly in facilitating acrosome formation and promoting germ cell proliferation and differentiation. Mechanistic studies have shown that exosomal miR-486-5p from Sertoli cells precisely regulates mouse SSC differentiation by modulating the Phosphatase and Tensin Homolog (PTEN) signaling pathway [92]. In parallel, testicular macrophages contribute to immune homeostasis and spermatogenic function through exosome secretion. Upon activation by Toll-like Receptor 4 (TLR4) agonists, the Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway is stimulated, leading to the release of exosomes enriched with Granulocyte Colony-Stimulating Factor (G-CSF) and Macrophage Inflammatory Protein-2 (MIP-2), which promote spermatogonial proliferation [93]. Exosomes derived from human Leydig cells contain multiple miRNAs, including miR-638, miR-149-3p, and miR-1246, which are closely associated with oligozoospermia and asthenozoospermia. Remarkably, these exosomes are capable of crossing the blood—testis barrier, allowing directional molecular transfer from the interstitial compartment to the seminiferous tubules [94]. Recent studies have also demonstrated that exosomes derived from bone marrow mesenchymal stem cells possess the capacity to induce germ cell differentiation [95], offering insights into potential therapeutic strategies for severe male infertility conditions such as azoospermia [96].

Seminal vesicle exosomes, first identified in human seminal plasma in 2009 [21], carry proteins, miRNAs, and bioactive lipids that may influence sperm motility, capacitation, and fertilization [97]. However, their molecular composition and functional mechanisms in humans remain poorly characterized, as most available evidence is derived from animal models. The lack of direct human validation limits the current understanding of their roles in fertility and infertility, highlighting a critical research gap.

Bulbourethral gland exosomes, secreted during sexual arousal and contributing to pre-ejaculatory fluid, represent the least studied component of SPE. Theoretical models suggest that these exosomes help regulate seminal fluid properties, including pH, ion homeostasis, and viscosity, thereby supporting sperm function. However, no specific proteins or miRNAs have been identified to date, and their effects on sperm motility, viability, or fertilization remain experimentally unvalidated. Technical challenges in isolating these low-abundance exosomes continue to hinder progress, thus emphasizing the need for advanced proteomic and transcriptomic approaches to clarify their biological roles.

6. Clinical validation of seminal plasma exosomal biomarkers

Seminal plasma exosomal biomarkers are promising biomarkers for the diagnosis of male infertility, offering improved accuracy and non-invasive alternatives to traditional semen analysis and invasive procedures such as testicular biopsy. TEX101 is a well-validated protein biomarker; in a study involving 805 seminal plasma samples, it demonstrated 100% sensitivity, 100% specificity, and an area under the curve (AUC) of 1.00 in distinguishing pre- and post-vasectomy men, thereby confirming its diagnostic value for azoospermia [98]. Exosomal microRNAs also show diagnostic potential. One study identified 57 differentially expressed miRNAs between normozoospermic and infertile individuals, providing molecular signatures associated with fertility status [99]. Among these, miR-31-5p achieved over 90% sensitivity and specificity in predicting the presence of sperm in testicular tissue and in distinguishing obstructive from non-obstructive azoospermia [100]. These exosomal biomarkers outperform conventional semen parameters and support more accurate, non-invasive fertility assessments.

7. Bibliometric keyword analysis of exosomes in male infertility

This section summarizes the relationship between keywords identified through bibliometric analysis and key research topics, and presents current research hotspots using a co-occurrence network generated by VOSviewer software (version 1.6.20, Centre for Science and Technology Studies, Leiden University, Leiden, The Netherlands). Data were obtained from the Web of Science Core Collection database, covering a period from 1970 to 2024. The search was conducted on 01 January 2024, using a targeted combination of search terms. For the “exosome” theme, terms such as exosomes, epididymosomes, and prostasomes were used and linked by the Boolean operator “OR”. For the “male reproductive system” theme, the terms male infertility, sperm, seminal plasma, semen, prostate, epididymis, and testis were used and similarly connected using “OR”. These two thematic groups were then combined using the operator “AND” to ensure the retrieval of literature relevant to both exosomes and the male reproductive system. This search strategy was specifically designed to capture articles closely aligned with the study’s research objectives.

The inclusion criteria focused on studies directly related to both the male reproductive system and exosomes. Studies were excluded if they lacked relevance to the male reproductive system; for example, those centered on prostate cancer or female reproduction, or if they involved non-human mammals and other animal models, including species such as horses, cattle, and Drosophila. These exclusions were necessary due to significant anatomical differences in the male reproductive tract across species. For instance, dogs and cats lack seminal vesicles, while the bovine prostate is largely undifferentiated. As a result, this review is restricted to exosomes associated with male infertility in the context of human reproductive biology.

After applying the inclusion and exclusion criteria, a total of 511 articles were retained for analysis. Keywords extracted from these articles were cleaned to eliminate terms directly related to the search queries, as well as any irrelevant or redundant words. Synonyms were merged, and keywords were standardized with respect to singular and plural forms and capitalization. The keyword co-occurrence analysis was conducted using VOSviewer software (version 1.6.20), and the resulting network is presented in Fig. 2.

SPE contain diverse components, including proteins and RNAs, which influence sperm motility, fertilizing capacity, and oocyte-binding ability. These molecules are involved

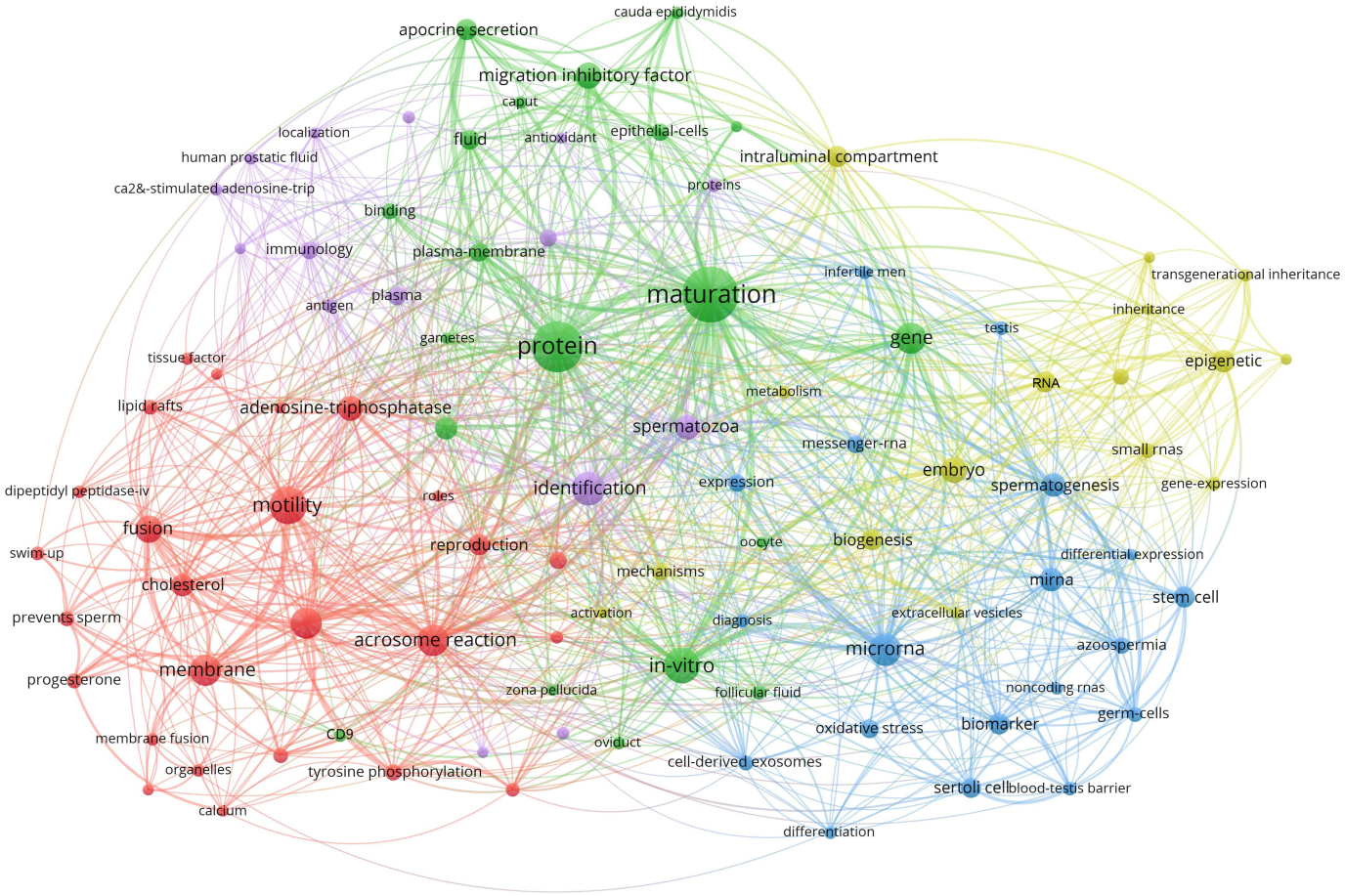

FIGURE 2. Keyword co-occurrence network in male reproductive system exosome research. Note: This network diagram visualizes keyword co-occurrence from 511 articles on exosomes in the male reproductive system, retrieved from the Web of Science Core Collection (1970–2024). The analysis includes terms related to exosomes (*e.g.*, epididymosomes, prostasomes) and male infertility (*e.g.*, sperm, testis, seminal plasma). Color-coded clusters represent major research themes: green (proteins, maturation, spermatozoa), red (motility, fusion, acrosome reaction), blue (miRNA, stem cells, spermatogenesis), yellow (gene expression, epigenetics, inheritance), and purple (immunology, fluid).

in key reproductive processes such as sperm maturation and sperm–oocyte interaction. As a result, the essential roles of SPE in the male reproductive system have attracted growing research interest [101].

Keyword analysis reveals that proteins currently occupy a central position in SPE-related research, with proteomic characterization identified as a major area of focus. Sperm maturation also emerges as a prominent hotspot, particularly in relation to how exosome-mediated signaling pathways regulate sperm development and function. Additionally, the co-occurrence network highlights interconnected nodes associated with genetic regulation and gene expression, including terms such as “gene”, “RNA”, and “microRNA”. These links suggest that RNA molecules within SPE, especially small RNAs such as miRNAs, may play important roles in modulating gene expression and influencing sperm function. The strong connections among the keywords “motility”, “membrane”, and “acrosome reaction” indicate that sperm motility is closely related to the biochemical properties of the sperm membrane and acrosomal responsiveness. This likely reflects the involvement of seminal plasma exosome-mediated signaling in regulating membrane structure and supporting motility-related functions.

8. Conclusions and outlook

Current male fertility assessments, which rely primarily on conventional semen analysis, account for only about 30% of infertility cases, underscoring the urgent need for more advanced diagnostic tools [102]. Although emerging methods, such as sperm DNA fragmentation assays and oxidative stress markers, are gaining acceptance, they remain insufficient to fully capture the complexity of male reproductive function [103]. Methodological limitations, including poor reproducibility, small sample sizes, and the lack of standardized protocols, further emphasize the need for novel and reliable biomarkers.

Seminal plasma exosomes (SPE) offer promising diagnostic and therapeutic potential in the field of male infertility [40]. Diagnostically, the protein and miRNA cargo contained within SPE provides a molecular fingerprint of reproductive health, enabling the development of non-invasive diagnostic kits for predicting sperm vitality, fertilization potential, and pregnancy outcomes with high accuracy. Therapeutically, while exosomes hold great potential, clinical translation remains limited. Currently, there are no registered clinical trials for SPE-based treatments for male infertility on ClinicalTrials.gov and

the World Health Organization—International Clinical Trials Registry Platform (WHO ICTRP). Existing evidence is limited to preclinical studies involving mesenchymal stem cell-derived exosomes. Translational barriers include inconsistent isolation and purification methods, lack of scalable manufacturing systems, undefined delivery and dosing strategies, limited safety evaluation, and evolving regulatory frameworks.

Among SPE subtypes, epididymosomes, prostasomes, and testicular exosomes each play distinct yet interconnected roles in male reproductive biology. Epididymosomes facilitate sperm maturation and capacitation through the transfer of proteins such as P34H and SPAM1, while also providing antioxidant protection via GPX5 and BLVRA. Prostasomes support post-ejaculatory capacitation and the acrosome reaction by delivering signaling molecules such as cAMP and Ca^{2+}, and enhance sperm survival in the female reproductive tract through immunomodulatory proteins like CD48. Testicular exosomes contribute to the regulation of spermatogenesis, with Sertoli cell-derived miR-486-5p modulating spermatogonial stem cell differentiation. Dysregulation of these exosomal pathways has been implicated in various male infertility subtypes, including oligozoospermia (linked to impaired spermatogenesis), asthenozoospermia (associated with motility and energy deficiencies), and teratozoospermia (resulting from sperm maturation abnormalities). Although exosomes from the seminal vesicles and bulbourethral glands remain underexplored, they may offer additional therapeutic opportunities in the future. Fig. 2 summarizes the types, origins, molecular cargo, functions, clinical relevance, and current research status of SPE. A detailed tabular summary of these exosome subtypes is provided in Table 2 (Ref. [16, 58, 61, 65, 68, 70–75, 87]).

SPE detection is non-invasive and utilizes its rich molecular cargo for personalized diagnostic and therapeutic strategies. However, reproducibility remains a major challenge due to small sample sizes and the absence of standardized protocols for exosome isolation and detection. Future studies could include the standardization of Good Manufacturing Practice-compliant protocols for exosome production, the execution of multicenter cohort studies to validate candidate biomarkers, the application of single-vesicle analysis and spatial omics to uncover mechanistic insights, and the development of targeted delivery systems, comprehensive safety assessments, and clearly defined regulatory pathways.

Technological advances, such as Artificial Intelligence (AI)-driven sperm classification, deep learning for multi-omics, exosome engineering, and nanotechnology-based detection, are expected to transform SPE research and accelerate its application in precision medicine [104]. The development of dual diagnostic pathways, combining biomarker identification with semen analysis, and therapeutic strategies from exosome production to targeted delivery, could be central to clinical translation. To support this progress, high-throughput detection platforms, curated exosomal cargo databases, and interdisciplinary collaboration among reproductive medicine, nanomedicine, and bioinformatics will be essential. Collectively, these efforts have the potential to transform the diagnosis and treatment of male infertility, and improve reproductive outcomes worldwide (Fig. 3).

TABLE 2. Summary of seminal plasma exosome subtypes based on current literature.

Exosome type	Origin	Key molecular cargo	Primary functions	Clinical relevance	Research status
Epididymosomes	Epididymal epithelial cells [58, 61]	Proteins: P34H [73], SPAM1 [70], PMCA4 [16], GPX5 [71], BLVRA [72], ADAM7 [74, 75], SLC27A2, EDDM3B, KRT19, WFDC8 [65], ELSPBP1 [72]; Other: miRNAs, mRNAs, lipids [68]	Sperm maturation, capacitation, oxidative stress protection, morphology and motility regulation	Associated with oligospermia, asthenospermia, and teratospermia	Well-established; hundreds of proteins identified
Prostasomes	Prostatic epithelial cells	PSA, PSCA, CD48 [87], transport proteins, signaling proteins, GTP-binding proteins	Post-ejaculatory capacitation, acrosome reaction, immune modulation in the female tract, sperm response to progesterone, antimicrobial action	Influences sperm motility, semen liquefaction, and immunosuppressio; relevant to fertilization	Well-characterized; extensive functional data
Testicular Exosomes	Sertoli cells, spermatogonia, Leydig cells, testicular macrophages	miRNAs: miR-486-5p, miR-638, miR-149-3p, miR-1246; Growth factors: G-CSF, MIP-2; Regulators: PTEN pathway modulators	Regulation of early spermatogenesis, SSC proliferation and differentiation, blood–testis barrier crossing, and immune balance	Linked to oligozoospermia and asthenozoospermia; potential application in azoospermia treatment	Emerging, recent mechanistic discoveries

TABLE 2. Continued.

Exosome type	Origin	Key molecular cargo	Primary functions	Clinical relevance	Research status
Seminal Vesicle Exosomes	Seminal vesicle epithelial cells	miRNAs, functional proteins, bioactive lipids	Regulation of sperm motility and fertilization capacity	Potential roles suggested, but no direct experimental validation	Limited; mainly animal data; human data lacking
Bulbourethral Exosomes	Bulbourethral gland epithelial cells	Not yet identified	Regulation of pH, ion concentration, and seminal viscosity	Theoretical functions only; no experimental validation	Virtually unexplored; isolation remains technically challenging

PSCA: prostate stem cell antigen; SPAM1: Sperm Adhesion Molecule 1; PMCA4: Plasma Membrane Calcium-Transporting ATPase 4; GPX5: Glutathione Peroxidase 5; BLVRA: Biliverdin Reductase A; ADAM7: A Disintegrin and Metalloproteinase Domain-Containing Protein 7; SLC27A2: Solute Carrier Family 27 Member 2; EDDM3B: Epididymal Protein 3B; KRT19: Keratin 19; WFDC8: WAP Four-Disulfide Core Domain Protein 8; ELSPBP1: Epididymal Sperm-Binding Protein 1; PSA: prostate-specific antigen; CD48: Cluster of Differentiation 48; G-CSF: Granulocyte Colony-Stimulating Factor; MIP-2: Macrophage Inflammatory Protein-2; PTEN: Phosphatase and Tensin Homolog; SSC: Spermatogonial stem cell.

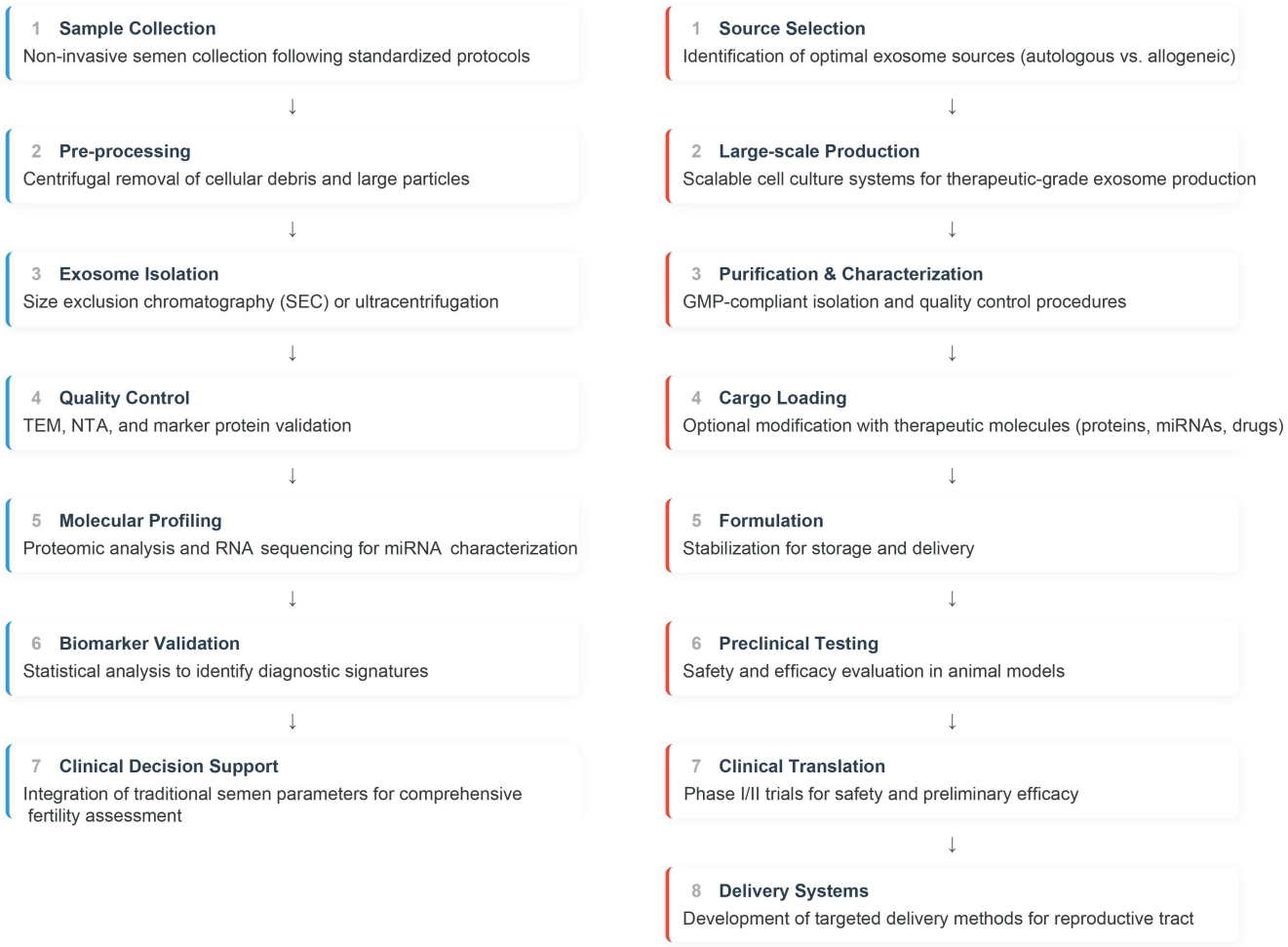

FIGURE 3. Diagnostic and Therapeutic Pathways for Seminal Plasma Exosome Research. Note: Illustration of dual pathways for advancing seminal plasma exosome research, encompassing both diagnostic and therapeutic strategies aimed at clinical application. TEM: transmission electron microscopy; NTA: nanoparticle tracking analysis; GMP: Good Manufacturing Practice.

ABBREVIATIONS

AI, Artificial Intelligence; AUC, area under the curve; cAMP, cyclic adenosine monophosphate; CD9/CD63/CD81/CD48, Cluster of Differentiation 9/63/81/48; DHT, dihydrotestosterone; ESCRT, endosomal sorting complex required for transport; G-CSF, granulocyte colony-stimulating factor; GMP, Good Manufacturing Practice; ILVs, intraluminal vesicles; MISEV, Minimal Information for Studies of Extracellular Vesicles; miRNA(s), microRNA(s); mRNA(s), messenger RNA(s); MIP-2, macrophage inflammatory protein-2; MVBs, multivesicular bodies; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, natural killer; NOA, non-obstructive azoospermia; NTA, nanoparticle tracking analysis; PEG, polyethylene glycol; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PTEN, phosphatase and tensin homolog; SEC, size exclusion chromatography; SPE, seminal plasma exosomes; SSC(s), spermatogonial stem cell(s); TEM, transmission electron microscopy; TLR4, Toll-like receptor 4; UC, ultracentrifugation; WHO ICTRP, World Health Organization International Clinical Trials Registry Platform; L-PGDS, Lipocalin-type Prostaglandin D Synthase; TEX101, Testis Expressed 101; ECM1, Extracellular Matrix Protein 1; DJ-1, Protein deglycase DJ-1; TKTL1, Transketolase-like 1; LDHC, Lactate Dehydrogenase C; PGK2, Phosphoglycerate Kinase 2; TSG101, Tumor Susceptibility Gene 101; GM130, Golgi Matrix Protein 130; SPAM1, Sperm Adhesion Molecule 1; PMCA4, Plasma Membrane Calcium-Transporting ATPase 4; SLC27A2, Solute Carrier Family 27 Member 2; EDDM3B, Epididymal Protein 3B; KRT19, Keratin 19; WFDC8, WAP Four-Disulfide Core Domain Protein 8; GPX5, Glutathione Peroxidase 5; BLVRA, Biliverdin Reductase A; ELSPBP1, Epididymal Sperm-Binding Protein 1; ADAM7, A Disintegrin and Metalloproteinase Domain-Containing Protein 7; ATPase, Adenosine Triphosphatase; MSC, mesenchymal stem cell.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

AUTHOR CONTRIBUTIONS

SJL and JG—designed the research study. ZZG and QJF—performed the research. EC—provided help and advice on language editing. FW, SJL and JG—analyzed the data and supervised the manuscript. ZZG and SJL—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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