Review

Seminal Plasma Extracellular Vesicles: Key Mediators of Inter-cellular Communication in Mammalian Reproductive Systems

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Review

Seminal Plasma Extracellular Vesicles: Key Mediators of Intercellular Communication in Mammalian Reproductive Systems

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Simple Summary: Seminal plasma extracellular vesicles have emerged as pivotal mediators of intercellular signaling within mammalian reproductive system by delivering bioactive signaling molecules to target cells. This review summarizes the emerging roles of seminal plasma extracellular vesicles as non-invasive diagnostic biomarkers for male fertility assessment and infertility diagnosis, while elucidating their regulatory effects on sperm maturation, sperm capacitation, and embryo implantation. The findings highlight the paramount importance of elucidating the molecular mechanisms through which seminal plasma extracellular vesicles mediate intercellular communication within reproductive systems. Such investigation is essential for improving breeding rate, and paving the way for novel therapeutic strategies targeting idiopathic infertility.

Abstract: Seminal plasma, traditionally regarded as a passive transport medium for sperm, has emerged as a sophisticated biofluid orchestrating critical dialogues in reproductive physiology. Contemporary research reveals its multifunctional role in modulating endometrial receptivity through molecular priming of the female reproductive tract, a process essential for successful embryo implantation. Notably, seminal plasma contains numerous extracellular vesicles, that serve as critical mediators of intercellular communication via the regulation of biological processes in target cells. Through this sophisticated vesicular communication system, seminal plasma extracellular vesicles (SPEVs) coordinate critical reproductive events. Thus, it will be important to elucidate the molecular mechanisms by which SPEVs mediate reproductive processes, to provide knowledge that may assist in infertility treatment. Herein, we elucidated the emerging potential of SPEVs as non-invasive diagnostic biomarkers for male fertility assessment and infertility diagnosis. Furthermore, this review systematically summarized current advances in SPEVs, highlighting their multifaceted roles in mediating sperm maturation, facilitating capacitation, and modulating embryo implantation through targeted delivery of bioactive signaling molecules.

Keywords: seminal plasma; extracellular vesicles; infertility; sperm maturation; sperm capacitation; embryo implantation

1. Introduction

The elevated incidence of early gestational failure represents a significant challenge in both livestock and clinical reproduction, with impaired embryo implantation accounting for majority of pregnancy loss [1,2]. Since its clinical inception four decades ago, in vitro fertilization has catalyzed revolutionary advancements in assisted reproductive technology, fundamentally improving breeding rate and transforming fertility treatment through innovative techniques including artificial insemination, gamete manipulation, intracytoplasmic sperm injection, and precision embryo transfer protocols [3]. Despite these achievements, the paternal contribution to reproductive abnormalities

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remains largely overlooked [4,5], primarily due to the persistent misconception that sperm merely function as genomic vectors for paternal DNA delivery.

Seminal plasma, a complex bioactive fluid synthesized through coordinated contributions from the testis, epididymis, vas deferens, and accessory glands (notably seminal vesicles and prostate), forms the microenvironment surrounding ejaculated sperm [6]. Emerging research has revealed that sperm delivery to the oocyte during conception represents only one facet of its biological functions. Current scientific evidence establishes that seminal plasma contains bioactive signaling molecules that exert significant regulatory effects on key reproductive processes, including sperm maturation, sperm capacitation and embryo implantation. During sperm transit through the female reproductive tract (FRT), seminal plasma plays a critical regulatory role in coordinating key reproductive processes: 1) maintaining sperm in decapitated state until optimal fertilization timing; 2) facilitating intravaginal sperm transport; 3) establishing oviductal sperm reservoir; 4) triggering precisely timed acrosome reactions; and 5) modulating gamete interactions through zona pellucida-binding proteins [7]. In addition, following seminal plasma exposure, cervical and uterine epithelial cells upregulate the secretion of proinflammatory cytokines (including GM-CSF, IL-1β, IL-6, and IL-8), which mediate the recruitment of neutrophils, macrophages, and dendritic cells (DCs) from peripheral circulation to both the endometrial stromal compartment and epithelial layer. This coordinated immune response facilitates endometrial receptivity establishment through stromal remodeling while simultaneously creating an immune-privileged microenvironment that safeguards sperm from immunological clearance [8]. However, the molecular mechanisms underlying the regulatory functions of bioactive signaling molecules in seminal plasma within the reproductive system require further elucidation.

Extracellular vesicles (EVs) are nano-sized, lipid bilayer-enclosed particles released by cells that transport bioactive signaling molecules such as proteins, lipids, and nucleic acids [9]. Notably, seminal plasma was among the earliest biological fluids where EVs were detected and characterized [10]. EVs have been extensively studied as critical mediators of intercellular communication, facilitating the transfer of bioactive signaling molecules (including proteins, lipids, and nucleic acids) from donor to recipient cells. Through mechanisms involving direct receptor stimulation and intracellular cargo delivery, EVs demonstrate remarkable capacity to modulate cellular functions and signaling pathways, making them pivotal targets for elucidating the molecular mechanisms underlying various biological processes [11]. Therefore, this review synthesizes current evidence regarding seminal plasma extracellular vesicles (SPEVs) as non-invasive diagnostic biomarkers for male infertility diagnosis, with particular emphasis on their cargo characterization and functional roles within reproductive systems, thereby highlighting critical knowledge gaps to guide future investigations.

2. Overview of Seminal Plasma Extracellular Vesicles

Initial observations of EVs biogenesis emerged from studies on reticulocyte maturation in 1987 [12]. Since then, EVs were detected across diverse biological fluids, with seminal plasma standing out as one of the earliest biofluids where EVs were systematically characterized [10]. It is noteworthy that seminal plasma contains a significantly higher concentration of EVs compared to most other bodily fluids. These EVs exhibit heterogeneous origins, primarily derived from the coordinated secretion of various organs in the male reproductive system: specifically, prostate (known as prostasomes, constituting 40% of SPEVs), epididymis (epididymosomes), along with seminal vesicles and testicles [13]. Due to the low abundance of seminal vesicle-derived and testicular-derived EVs in seminal plasma, there is limited research in this area. Recently, a new non-contact isolation protocol for Tissue EVs has been reported, which employing immunomagnetic separation to specifically deplete undesired non-Tissue EVs [14]. The implementation of this methodology significantly enhances isolation purity by minimizing co-isolated contaminants, thereby greatly contributing to the progress of seminal vesicles- and testicles- derived EVs research.

Prostasomes are bilamellar to multilamellar membrane-bound vesicles measuring 30-500 nm, are predominantly secreted by prostate epithelial cells into the acinar lumen and constitute the major

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component of SPEVs [15]. Emerging evidence has revealed two distinct subpopulations of prostasomes classified by size and molecular composition: 1) small vesicles enriched with glioma pathogenesis-related 2 (GLIPR2) and 2) larger vesicles demonstrating annexin A1 (ANXA1) predominance. However, current research has not yet elucidated the functional differentiation between these two prostasome populations [16]. Prostasomes play a pivotal role in regulating sperm motility and orchestrating the precise timing of the acrosome reaction, primarily mediated through the transfer of Ca^{2+}-signaling receptors to the neck region of ejaculated sperm [17]. Mechanistically, prostasome fusion delivers three critical components to sperm: 1) progesterone receptors, 2) cyclic adenosine diphosphoribose (cADPR)-synthesizing enzymes, and 3) ryanodine receptors (RyRs). This coordinated delivery regulated Ca^{2+} elevation establishes within sperm, which precisely modulate flagellar hyperactivation, thereby underpinning the sperm's fertilization competence essential for successful gamete fusion [18]. Furthermore, prostasomes also influence sperm capacitation through cAMP-dependent activation of protein kinase A (PKA) [19]. Furthermore, as sperm migrate through the vagina, cervix, uterus, and oviduct, prostasomes interaction in the FRT suppress female immune responses to sperm by inhibiting the phagocytic activity of monocytes and neutrophils, and reducing natural killer (NK) cell activity [20].

Epididymosomes exhibit a polydisperse size distribution (25-300 nm) with membrane enriched in cholesterol-sphingomyelin lipid rafts that are essential for protein transfer between epididymosomes and sperm. In addition, Epididymosomes constitute a relatively small proportion of SPEVs in ejaculated semen, indicating their primary functional role in sperm maturation and membrane stabilization during epididymal transit rather than post-ejaculation [21]. Similar to prostasomes, epididymosomes are proposed to exist as two distinct subpopulations: epididymal sperm binding protein 1 (ELSPBP1)-enriched epididymosomes and CD9-positive epididymosomes. ELSPBP1-enriched epididymosomes are believed to protect epididymal sperm from oxidative stress through an antioxidant cycle. Specifically, these specialized vesicles form a functional complex with biliverdin reductase A (BLVRA), catalyzing the NADPH-dependent reduction of biliverdin to bilirubin. The biliverdin acts as an endogenous antioxidant by effectively scavenging reactive oxygen species (ROS) from immature sperm, thereby protecting maturing sperm. Simultaneously, bilirubin undergoes Zn^{2+}-dependent reconversion to biliverdin, completing a redox cycle that sustains antioxidant defense mechanisms [22]. CD9-positive epididymosomes are postulated to orchestrate critical mammalian sperm maturation processes during epididymal transit. It exhibits temperature- and pH-dependent binding and fusion with sperm, mediating targeted protein delivery to post-acrosomal sheath and midpiece domains. This process facilitates mammalian sperm maturation through multiple mechanisms: 1) regulation of Ca2+ channel gating, 2) enhancement of zona pellucida binding affinity, 3) activation of progressive motility, and 4) suppression of premature acrosome reaction [23].

3. Harnessing Seminal Plasma Extracellular Vesicles Contents as Non-Invasive Diagnostic Biomarkers for Livestock Fertility Assessment and Male Infertility Diagnosis

In livestock production, artificial insemination technology has been extensively utilized for livestock breeds improvement [24]. The precise evaluation and selection of high fertility male livestock for insemination are critically important to enhance conception. However, contemporary evaluation of male livestock fertility predominantly relies on semen quality assessment. Current research indicates that the diagnostic accuracy of these conventional techniques remains suboptimal [25], which significantly limited the application of artificial insemination. Moreover, azoospermia and oligoasthenoteratozoospermia are recognized as predominant causes of male infertility, the underlying male etiology remains undetermined in approximately 70% of infertile couples [26]. In addition, prostate cancer, the second most prevalent malignancy in men worldwide, posing a significant threat to men's health and quality of life. According to 2020 global estimates, this disease claimed 375,304 lives worldwide [27]. This deficiency stems partly from the lack of reliable non-

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invasive diagnostic tools. Current clinical practice predominantly relies on tissue biopsy analyses, which faces significant limitations due to tissue heterogeneity and inherent challenges in sampling techniques, frequently yielding inconclusive results. In this context, the identification of specific non-invasive biomarkers represents a critical priority in both accurate selection of livestock with high fertility and advancing therapeutic strategies for male infertility.

Since EVs have a lipid bilayer structure, the proteins and nucleic acids they carried are stable in body fluids [28]. Additionally, EVs contain molecules of the progenitor cell, so these EVs in the fluids can reflect the identity, characteristics, and health of the cell or tissue of origin [13]. Due to these attributes, the contents of EVs are considered relevant for study as reliable biomarkers. Specifically, in recent years, an increasing number of studies have been published that evaluate the contents of SPEVs in fluids as diagnostic biomarkers for livestock fertility assessment and male infertility diagnosis (Table 1).

Notably, accumulating evidence has demonstrated that Non-coding RNA (ncRNA) are emerging diagnostic biomarkers due to its crucial biological significance through transcriptional and post-transcriptional modifications [29]. Among them, circRNA exhibit multifaceted regulatory functions in cellular processes, including regulate transcription, promoting DNA breaks, inhibiting RNA binding protein activity, acting as an enhancer or scaffold for various proteins, being translated into functional peptide, and interfering mRNA function [30]. In addition, circRNA serve as competitive endogenous RNA (ceRNA) to sponge miRNA, forming regulatory networks with lncRNA and mRNA [31,32]. Since this ceRNA network involves multiple RNA, which provides a multidimensional framework for elucidating complex biological processes in reproductive biology. Thereby, in-depth investigation of ceRNA network dynamics in SPEVs holds promise for improving fertility assessment in livestock production and advancing diagnostic strategies in male infertility.

Table 1. Summary of seminal plasma extracellular vesicles contents as diagnostic biomarkers for livestock fertility assessment and male infertility diagnosis in last five years.

Phenotype	Species	subtype	biomarker	Reference
Fertility	bull	protein	SP10, ADAM7, and SPAM 1	[33]
		miRNA	miR-195	[34]
	boar	protein	EZRIN	[35]
		miRNA	miR-26a	[36]
	buffalo	protein	PDIA4 and GSN	[37]
	rabbit	miRNA	miR-190b-5p, miR-193b-5p, let-7b-3p, and miR-378-3p	[38]
Sperm motility	boar	gene-lipid linkages	CerG1 (d22:0/24:0) - RCAN3, Cer (d18:1/24:0) - SCFD2 and CerG1 (d18:0/24:1) - SCFD2	[39]
		protein	GART, ADCY7, and CDC42	[40]
		miRNA	miR-122-5p, miR-486, miR-451, miR-345-3p, miR-362, and miR-500-5p	[41]
		miRNA	miR-205, miR-493-5p, and miR-378b-3p	[42]
		miRNA	miR-222	[43]
		circRNA	circCREBBP	[44]
	buffalo	protein	ACRBP, SPACA1, PRDX5, SPACA4, DYNLL2, ZAN, IZUMO1, and ADAM2	[45]
Conception rates	boar	protein	GPX5	[46]
Semen quality	human	protein	LTF, CRISP3, SERPINA3, ELSPBP1, GSTM3, AGP2, SAP, ANPEP, MME, and FAS	[47]
		miRNA	miR-10b-3p, miR-122-5p, miR-205-5p, miR-222-3p, miR-34c-5p, miR-509-3-5p,	

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		miRNA	miR-888-5p, miR-892a, miR-363-3p, miR-941, miR-146a-5p, and miR-744-5p	[48]
		miRNA	miR-7110, miR-4800, miR-4488, miR-3916, and miR-4508	
		circRNA	hsa_circ_0009013, hsa_circ_0123184, hsa_circ_0114168, hsa_circ_0139507, and hsa_circ_0139505	
		piRNA	piR-hsa-26399, piR-hsa-28160, piR-hsa-28478, and piR-hsa-1077	
		rRNA	URS00008C6BF7, URS00008C9E2E, URS0000914753, URS0000CA0D60, and URS00008CE4BC	
		lncRNA	URS0000D56E09, URS0000D5AE24, URS0000A7764F, ENST00000631211.1, and ENST00000629969.1	
Live birth rate	human	circRNA	hsa_circ_0103367, hsa_circ_0008611, hsa_circ_0008109, hsa_circ_0004177, hsa_circ_0009684, hsa_circ_0013829, hsa_circ_0035429, hsa_circ_0114168, hsa_circ_0001488, and hsa_circ_0118471	[49]
		piRNA	piR-hsa-28478 and piR-hsa-1077	
Azoospermia	human	miRNA	miR-10a-5p, miR-146a-5p, miR-31-5p, miR-181b-5p	[50,51]
Non-obstructive azoospermia	human	tsRNA	tRF-Val-AAC-010 and tRF-Pro-AGG-003	[52]
Oligoasthenospermia	human	circRNA	has_circ_0004721, has_circ_0002452, has_circ_0079245, has_circ_0005584, has_circ_0003823, has_circ_8826, has_circ_0125759, has_circ_0109282, and has_circ_0009142	[53]
Spermatogenic ability	human	piRNA	piR-has-61927	[54]
		protein	ANXA2 and KIF5B	[55]
Unilateral varicocele	human	miRNA	miR-210-3p	[56]
Prostate cancer	human	protein	KLK3, KLK2, MSMB, NEFH, PSCA, PABPC1, TGM4, ALOX15B, and ANO7	[57]
		protein	CRP and H2B2E	
		mRNA	*CASP3*, *DDX11*, *DLC1*, *ETV1*, *PTGS1*, *TP53*, and *VEGF*	[58]
		miRNA	miR-141-3p	
		miRNA	miR-27a-3p, miR-27b-3p, miR-155-5p, and miR-378a-3p	[59]
		tsRNA	5'-tRNA-Glu-TTC-9-1_L30 and 5'-tRNA-Val-CAC-3-1_L30	[60]

4. Seminal Plasma Extracellular Vesicles Promote Sperm Maturation

Mammalian sperm exhibit transcriptional and translational quiescence due to their highly condensed chromatin structure [61], indicating that post-testicular maturation events in the epididymis and female reproductive through external signals such as SPEVs are particularly important.

During epididymal maturation, sperm migrate through the three functionally distinct epididymal segments (caput, corpus, and cauda), each exhibiting unique transcriptional and

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proteomic profiles that drive region-specific sperm remodeling [62,63]. The caput epididymis maintains the most abundant and diverse secretory profile, where a dynamic molecular exchange occurs: testicular-derived proteins from sperm undergo rapid absorption while epididymal-specific proteins are actively secreted. This sophisticated molecular reprogramming ultimately endowing sperm with two critical functional competencies: swimming in a progressive manner and capacity for oocyte recognition [64]. These functional characteristics progressively mature in the corpus epididymis before attaining their peak functional capacity for motility and fertilization in the distal caudal segment [65]. In this process, sperm undergo extensive physiological remodeling mediated through the transfer of proteins and lipids via epididymosomes, including progressive sphingomyelin accumulation and cholesterol depletion [66], membrane rigidity reduction [67], spatial redistribution of surface antigens [68], structural stabilization through increased disulfide bond formation [69], and coordinated surface protein modification through selective removal, addition, and post-translational processing [70].

Notably, epididymosomes also mediate intercellular communication by delivering a heterogeneous population of small non-coding RNAs (sncRNAs), including miRNAs and tRNA, to maturing sperm. This transfer dynamically remodels the sperm sncRNA profile, potentially regulating post-transcriptional gene expression and contributing to paternal epigenetic inheritance during post-testicular maturation [71,72]. In addition, these sncRNA are subsequently translocated into the oocyte during gamete fusion, where they establish epigenetic regulation of embryo development through modulation of a specific subset of genes [71]. These findings elucidate the multifaceted roles of epididymosomes in mammalian reproduction, delineating the molecular mechanisms underlying cargo delivery to recipient cells (oocytes or endometrial epithelial cells) and providing mechanistic insights into their regulatory functions during embryo development and implantation processes. However, research progress has been limited by technical challenges in obtaining high-purity epididymal sperm and epididymosomes. Recent methodological advancements, including the development of novel Tissue EVs isolation protocols and the establishment of animal models for comparative biomarker discovery [73], show potential to significantly advance this field. It is noteworthy that epididymosomes exhibit regional heterogeneity in both size and molecular composition along the epididymal segments [74]. These emerging methodologies could facilitate systematic characterization of molecular mechanisms by which epididymosomes mediate post-testicular sperm maturation processes.

5. The Regulatory Role of Seminal Plasma Extracellular Vesicles in Sperm Function

Following ejaculation, SPEVs exert multifaceted regulatory effects on sperm viability and function through both direct and indirect mechanisms. Direct regulation manifests through their involvement in critical physiological processes including sperm motility enhancement, capacitation initiation, and acrosome reaction, while indirect protective functions are achieved through microenvironmental stabilization for sperm within the FRT.

Sperm motility constitutes a fundamental determinant of natural fertility, particularly for ensuring successful post-ejaculatory survival and functionality within the FRT. Recent researches have demonstrated that SPEVs enhance sperm progressive motility parameters by providing bioenergetic support through ATP synthesis [75] and modulating intracellular Ca^{2+} concentrations via CatSper-mediated calcium signaling [76]. In addition, advancements in bioinformatic analysis and next-generation sequencing platforms have catalyzed groundbreaking discoveries in SPEVs research, particularly regarding their molecular cargo and functional implications for sperm motility. Specifically, GPX5 significantly enhances sperm motility through elevation of total antioxidant capacity of sperm [46], while miR-222 exhibits pronounced motility-promoting effects via targeted suppression of BCL2L11-mediated apoptotic pathways [43]. Furthermore, circCREBBP improves sperm motility via the PI3K-Akt signaling pathway through competitive binding miR-10384 and miR-143-3p [44]. Collectively, these findings advance our understanding of the molecular pathways

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through which SPEVs regulate sperm motility, highlighting the functional importance of SPEVs-contained protein and non-coding RNAs (ncRNAs).

Sperm capacitation serves as a critical physiological prerequisite for successful fertilization, during which sperm acquire the ability to undergo the acrosome reaction - an exocytotic event essential for zona pellucida penetration and subsequent fusion with the oocyte plasma membrane [77]. Notably, the timing of this process must be tightly regulated within the FRT, as premature or dysregulated capacitation may lead to premature acrosome reaction and subsequent sperm degeneration, ultimately compromising fertilization potential [78]. Emerging evidence suggests that SPEVs inhibit sperm capacitation through selective packaging of bioactive cargo, including cytoskeletal protein EZRIN [35] and ncRNAs such as miR-21-5p [79]. Although the direct regulatory effects of SPEVs on sperm capacitation have been extensively documented, conflicting findings persist in this research domain. Pons-Rejraji et al. [80] identified a transient upregulation of tyrosine phosphorylation in sperm proteins during SPEVs treatment, paradoxically culminating in partial capacitation inhibition following prolonged incubation (3 hours). This biphasic regulation contrasts with reports by Bechoua et al. [81], who documented sustained downregulatory effects on tyrosine phosphorylation patterns. The complexity deepens with Murdica et al.'s demonstration that sperm exposure to SPEVs enhanced tyrosine phosphorylation levels and promoted acrosome reaction [82]. Notably, Barranco et al. [83] and Tamessar et al. [84] reported null effects of SPEVs treatment, finding neither alteration in capacitation status nor acrosome reaction. The observed discrepancies may be attributed to multiple methodological variations, particularly the diversity of isolation methods that differentially impact both sample purity and yield, coupled with the inherent heterogeneity in SPEVs morphology and molecular composition [85]. Additionally, biological variables including species-specific characteristics, age, physiological status, and environmental conditions may act as confounding variables contributing to apparent discrepancies in experimental outcomes. While previous studies have revealed the crucial involvement of SPEVs in sperm motility and capacitation, significant gaps remain in elucidating their molecular mechanisms. Comprehensive investigations are needed to characterize the complex functional roles and regulatory networks through which SPEVs modulate critical sperm functions.

6. Function of Seminal Plasma Extracellular Vesicles in Female Reproductive Tract

Emerging evidence reveals that SPEVs play a critical role in mediating sperm survival, sperm-egg binding and embryo implantation through modulating immune system and establishing the endometrial receptivity during sperm transit in the FRT.

The FRT maintains a unique immune regulatory microenvironment through localized immunomodulatory mechanisms. Establishment of immune tolerance toward allogeneic sperm and semi-allogeneic conceptus constitutes a critical prerequisite for successful fertilization and pregnancy maintenance [86]. This specialized immunotolerant milieu is supported by distributed professional antigen-presenting cells (APCs) across FRT, encompassing macrophages, DCs, and MHC class II-expressing epithelial populations. Notably, sperm- and conceptus-derived antigens preferentially drive peripheral regulatory T cell (Treg) differentiation rather than effector T cell activation, thereby establishing active immune tolerance through Treg-mediated immunosuppressive functions [87]. Disruption of the effector T cell/Treg equilibrium correlates with clinical manifestations ranging from impaired fertility and gestational complications to heightened infection susceptibility, reflecting the dual imperatives of FRT immunity: balancing pathogen defense with reproductive tolerance [88]. Notably, SPEVs have been demonstrated to induce the secretion of pro-inflammatory cytokines (particularly IL-6 and IL-8) [89,90], promote DCs maturation [91], and subsequently drive naive T cells differentiated into Tregs [92]. Mechanistically, IL-6 serves as a pivotal mediator in pregnancy-related immune adaptation, where it not only regulates maternal-fetal immunological tolerance but also mediates critical processes during embryo implantation through orchestrating the targeted migration of trophoblast cells to the decidual interface [93]. Furthermore, IL-8 functions as a potent

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chemokine that recruits peripheral monocytes possessing the capacity to differentiate into DCs [94]. Concomitantly, DCs actively participate in the differentiation of naive T cells into Tregs. Compelling evidence from murine models reveals that depletion of uterine DCs during the implantation window results in disrupted vessel formation and subsequent embryo implantation failure. In addition, uterine DCs mediate maternal-fetal immune tolerance by phagocytosing sperm-derived alloantigens and facilitating their cross-presentation to paternal antigen-specific T cells [95,96]. In addition, SPEVs attenuate natural killer (NK) cell-mediated cytotoxicity against sperm through CD48-CD244 receptor-ligand interaction, where CD48 molecules in SPEVs engage with the activating receptor CD244 expressed on NK cells, thereby shielding sperm from immune-mediated destruction [97].

The establishment of receptive endometrium is essential for successful embryo implantation and pregnancy. Contemporary research estimates that suboptimal endometrial receptivity underlies approximately 67% of implantation failure cases, positioning it as the predominant etiological factor in recurrent implantation failure [98]. In 2014, Vojtech et al. characterized the small RNA expression profile of SPEVs, proposing their potential regulatory role in facilitating the establishment of endometrial receptivity prior to embryo implantation through regulating endometrial cell proliferation and inducing the expression of immune-related genes in the endometrium [13]. Subsequent studies have substantiated this regulatory paradigm. Notably, EVs derived from healthy donors' seminal plasma significantly enhance endometrial receptivity through multiple mechanisms: 1) inducing in vitro decidualization of human endometrial stromal cells with concomitant prolactin secretion [99]; 2) upregulating receptivity-related molecular markers (MUC1, LIF, G-CSF, CX3CL1, VEGF) in endometrial epithelial cells [100]. Conversely, SPEVs from infertile patients exhibit inhibitory effects, suppressing both endometrial receptivity formation and marker gene expression [101]. Mechanistic studies further demonstrate that fertile SPEVs facilitate trophoblast-endometrial adhesion via LIF-STAT3 signaling pathway activation, ultimately promoting successful embryo implantation [102]. These findings collectively elucidate the crucial role of SPEVs in modulating the endometrial microenvironment for successful pregnancy establishment.

7. Conclusions

Emerging evidence underscores the pivotal role of SPEVs in modulating reproductive systems. While the precise molecular mechanisms through which SPEVs contribute to infertility and associated reproductive dysfunctions remain incompletely characterized, these nanoscale vesicles establish a novel paradigm for enhancing reproductive outcomes through improved implantation rate prediction and novel therapeutic target. The continued advancement of microfluidics platforms enables integrated extracellular vesicle isolation and biomarker analysis with enhanced precision, offering clinicians non-invasive diagnostic tools through SPEVs contents profiling while simultaneously advancing mechanistic studies of SPEVs-mediated regulatory pathways. Furthermore, elucidating the mechanisms by which SPEVs mediate targeted cargo transport to recipient cells could lead to the identification of novel therapeutic targets amenable to pharmacological intervention for infertility.

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