Extracellular Vesicles in Veterinary Medicine: Biological Foundations, Diagnostic Opportunities, and Therapeutic Advances

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ABSTRACT

Extracellular vesicles (EVs), membrane-enclosed nanoparticles, are pivotal mediators of intercellular communication with transformative potential in human and veterinary diagnostics and therapeutics. In veterinary medicine, EVs are increasingly recognized as minimally invasive biomarkers and next-generation biologics for regenerative medicine, immunomodulation, drug delivery, and oncology.

This review synthesizes the biological properties of EVs—including biogenesis, molecular composition, and cargo mechanisms—and evaluates current isolation and characterization techniques. Furthermore, it examines the diagnostic utility of EV-derived biomarkers and explores diverse therapeutic avenues, such as mesenchymal stromal cell-derived, milk-derived, and pathogen-associated EVs, alongside engineered platforms for targeted interventions.

By addressing current barriers and identifying research priorities, this article outlines the transition of EV science from experimental discovery to evidence-based implementation in precision veterinary medicine.

Keywords: Extracellular vesicles, veterinary medicine, EV-derived biomarkers, diagnosis, regenerative medicine

1. INTRODUCTION

Extracellular vesicles (EVs) have evolved from being viewed as inert cellular debris to recognized as vital mediators of intercellular communication [1,2]. EVs secreted by nearly all cell types into extracellular environment and facilitate complex signaling by transporting diverse molecular cargos—including proteins, lipids, and nucleic acids—across physiological and pathological states [1,3]. Their capacity to orchestrate critical biological processes, such as immune regulation, tissue repair, and tumor progression, has established them as significant subjects for translational research in both human and veterinary medicine [2,4].

The rising interest in EVs within veterinary science stems from the urgent need for advanced diagnostic biomarkers and innovative therapeutic modalities. Unlike traditional clinical methods, EVs serve as highly informative liquid biopsy platforms, as their molecular cargo reflects the physiological state of their cells of origin [5,6]. Furthermore, their nanoscale size, biological stability, and intrinsic biocompatibility make them promising candidates for regenerative and immunomodulatory therapies [7,8]. Given their presence in diverse biological fluids— including blood, milk, urine, saliva, semen, follicular fluid, amniotic fluid, broncho-alveolar lavage fluid, synovial fluid, and etc., —EVs offer significant potential for both early disease detection and targeted drug delivery in both livestock and companion animals [1,6, 46, 9].

A defining characteristic of EVs is their inherent biological diversity. While EVs are traditionally categorized into distinct subtypes based on their biogenesis—including exosomes, microvesicles, and apoptotic bodies—this classification is increasingly viewed as an oversimplification [1,3]. Emerging evidence suggests significant heterogeneity and overlap in the physical and molecular profiles of these populations. Consequently, the biological interpretation of EV-related data is highly dependent on the specific methodologies employed for their isolation, enrichment, and characterization [2,18].

2. BIOLOGICAL BASIS of EVs

2.1 EVs classification

As mentioned above, EVs were grouped into exosomes, microvesicles, and apoptotic bodies based on size, biogenesis routes, and Internal contents [1,6] (Table 1). Exosomes are often described as small EVs (30-150 nm) generated within the endosomal system and released following fusion of Multivesicular bodies (MVBs) with the plasma membrane; microvesicles (<1000 nm, ectosomes, or microparticles) are formed by outward budding and fission of the plasma membrane; apoptotic bodies (<5000 nm) are released during programmed cell death (apoptosis) and can contain organelle fragments and nuclear material [1,6,7]. However, size alone is insufficient for definitive subtype assignment because isolation methods enrich mixtures of vesicles and non-vesicular nanoparticles, and because the physical properties of EVs overlap substantially, especially in complex veterinary fluids rich in proteins and lipoproteins [2,3,8].

To overcome the limitations of biogenesis-based classification, current veterinary research is increasingly adopting a pragmatic approach centered on measurable physical and biochemical attributes. By defining preparations based on size (e.g., small versus medium/large EVs), density, and specific isolation workflows, researchers can significantly enhance the reproducibility of their findings [2,3,8]. This shift is particularly vital in livestock research, where complex biofluids—such as milk and plasma—often contain confounding co-isolates like casein micelles and lipoproteins. Rigorously defining preparations according to these metrics allows for a more transparent assessment of study results and mitigates the risk of experimental bias in biomarker discovery and functional testing [3,8,9].

2.2 Biogenesis pathways and secretion mechanisms

EV biogenesis can be broadly separated into endosomal-derived small EV formation and plasma membrane-derived vesiculation, with additional vesicle-like particles released during cell stress and apoptosis [1,6,7]. Although the molecular machinery is best characterized in human and murine systems, the core mechanisms appear conserved across mammals and are increasingly being explored in veterinary species in the contexts of inflammation, reproduction, infection, and cancer [1,4,10].

2.2.1 Endosomal pathway: exosomes formation pathway

In the endosomal pathway, early endosomes mature into late endosomes that contain inward budding of the endosomal membrane to form intraluminal vesicles (ILVs). When these endosomes become MVBs, they face two principal fates: fusion with lysosomes for cargo degradation or fusion with the plasma membrane for ILV release as small EVs. Sorting of specific proteins and nucleic acids into ILVs involves coordinated actions of ESCRT (endosomal sorting complexes required for transport) components and ESCRT-independent mechanisms, including roles for tetraspanins, ceramide-rich membrane domains, and lipid remodeling pathways [6,11,12] (Figure 1). These exosomes are characterized by specific internal factors inherent to these nano-sized structures. The balance between MVB degradation and secretion is sensitive to cellular state (e.g., hypoxia, inflammation, oncogenic signaling, infection), creating a mechanistic basis for why EV abundance and cargo composition track disease activity and therapeutic response [4,10,12].

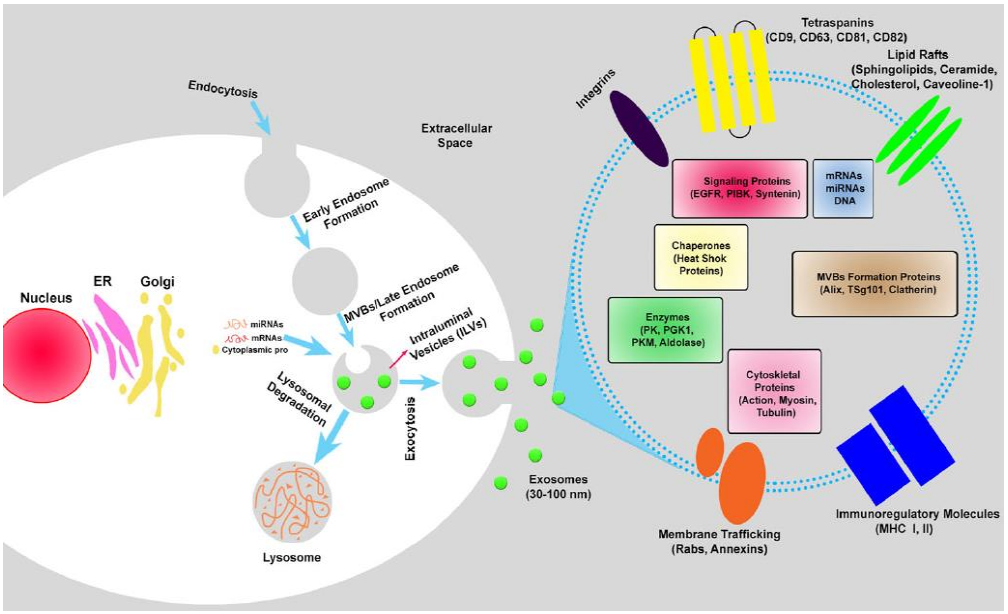

Figure 1: *Schematic illustration of the exosome formation process. This pathway involves three steps: (1) formation of early endosomes, (2) their maturation into multivesicular bodies (MVBs) coupled with the generation of intraluminal vesicles (ILVs), and (3) the fusion of MVBs with the plasma membrane to release exosomes into the extracellular environment.*

2.2.2 Plasma membrane budding: microvesicles formation

Microvesicles (MVs) form by outward budding from the plasma membrane and are linked to local remodeling of membrane lipid asymmetry, cytoskeletal reorganization, and regulated membrane scission [6,7]. Cellular activation, shear stress, inflammation, and oncogenic transformation can all increase membrane blebbing and MVs release. In veterinary inflammatory disease settings such as mastitis, these pathways are relevant because activated immune cells and stressed epithelial cells can markedly increase EV output, shaping local immune signaling and potentially altering milk EV profiles detectable for diagnostics [4,13].

2.2.3 Apoptotic body release and stress-associated vesiculation

During apoptosis, cells fragment into larger vesicular structures that can carry DNA, histones, and organelle components, and may influence immune recognition and clearance. While apoptotic bodies are sometimes discussed separately from EVs, many routine isolation pipelines (especially low-specificity methods) can co-enrich apoptotic bodies or their fragments, which is critical to acknowledge in veterinary samples collected from inflamed or necrotic tissues and from tumors where cell turnover is high. Stress-associated vesiculation also occurs during senescence, oxidative stress, or pathogen challenge, potentially producing particles that share features with EVs but have distinct compositions and functions [7,10,14].

2.3 EVs contents and functional heterogeneity

The molecular cargo encapsulated within EVs varies significantly depending on their biogenesis pathways, cellular origin, and environmental conditions. Exosomes, derived from the endosomal pathway via MVBs, typically contain a highly selective enrichment of molecular components. Their cargo is characterized by various RNA species (e.g., mRNA, miRNA, and lncRNA), along with specific protein markers such as tetraspanins (CD9, CD63, CD81), Alix, TSG101, and heat shock proteins. Additionally, exosomes harbor specific lipids and metabolic enzymes, reflecting their specialized role in mediating precise intercellular communication and regulatory signaling. In contrast, MVs and apoptotic bodies exhibit more heterogeneous compositions. MVs, which originate from the direct outward budding of the plasma membrane, often carry a higher proportion of membrane proteins, adhesion molecules, and signaling receptors derived from the parent cell surface, alongside cytosolic enzymes. Apoptotic bodies, formed during programmed cell death, possess a distinct profile typically comprising nuclear fragments, cellular organelles, and cytoplasmic debris. These variations in internal content not only serve as a reflection of the distinct formation mechanisms of each EV

subtype but also provide essential criteria for their isolation, characterization, and functional analysis in biomedical research [1,6,11, 12]. In veterinary medicine like human medicine, EV cargos is particularly important for biomarker discovery and therapeutic applications, as it can reflect tissue-specific pathology and deliver regenerative, immunomodulatory, or engineered molecular signals [4,5,15].

The functional diversity of EVs results from differences in donor cell type, cellular stimuli, and the complexity of biofluids. Distinct cells release EVs with unique surface markers that influence targeting and uptake, while the same cell type may produce different EV subpopulations under conditions such as infection, hypoxia, or homeostasis [10,12]. Moreover, biological fluids often contain mixed EV populations and contaminants, such as lipoproteins in blood or casein-related structures in milk, which may interfere with interpretation. Therefore, rigorous EV characterization and control of veterinary-specific confounders—including diet, lactation stage, estrous cycle, stress, and sampling conditions—are essential for reliable diagnostic and therapeutic conclusions [3,5,16].

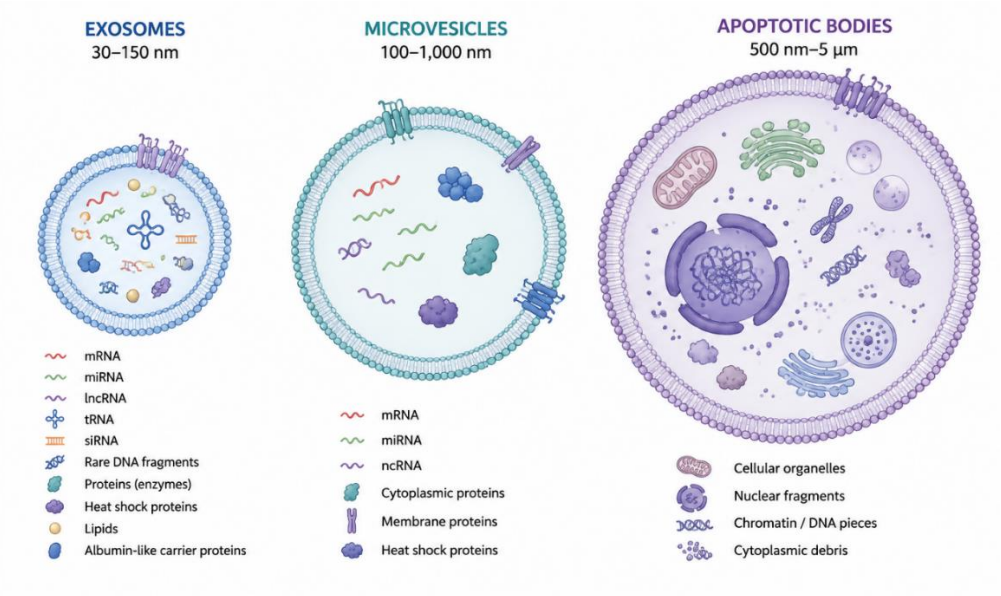

Figure 2: *Intracellular Cargo of Extracellular Vesicle Subpopulations.*

2.4 Uptake mechanisms of EVs and modes of action in recipient cells

EV-mediated signaling depends on the interaction of extracellular vesicles with recipient cells through surface binding, internalization, membrane fusion, or a combination of these processes, followed by cargo delivery and modulation of downstream cellular pathways [1,6]. EVs uptake is mediated by several mechanisms, including receptor–ligand interactions, clathrin- and caveolin-dependent endocytosis, lipid raft–mediated uptake, macropinocytosis, phagocytosis—particularly in professional phagocytes such as macrophages and dendritic cells—and, under specific conditions, direct fusion with the plasma membrane [6,12]. The efficiency, specificity, and biological consequences of EV uptake are influenced by EV surface molecules, including integrins, tetraspanins, phosphatidylserine, glycans, and adhesion molecules, as well as by recipient cell phenotype, tissue microenvironment, inflammatory status, and the presence of opsonins, complement components, or immune complexes. These factors collectively determine EV tropism, intracellular trafficking, cargo release, and the ultimate functional response of the recipient cell.

In immune and inflammatory contexts relevant to veterinary medicine, EVs can modulate antigen presentation, cytokine secretion, leukocyte recruitment, and macrophage polarization, thereby shaping host responses during infection, tissue injury, and chronic inflammatory disease [10,15]. In reproductive tissues, EVs function as mediators of paracrine and endocrine-like communication, coordinating gamete function, embryo–maternal crosstalk, and early developmental competence. EVs present in seminal plasma, uterine fluid, follicular fluid, and embryo culture media have been associated with regulation of sperm motility and capacitation, endometrial receptivity, embryo implantation, and early embryonic development [16–18]. In oncology, tumor-derived EVs contribute to tumor progression by remodeling the tumor microenvironment, suppressing antitumor immunity, promoting angiogenesis, enhancing invasion, and preparing premetastatic

niches. Conversely, engineered or therapeutic EVs may be harnessed to counteract these processes through the delivery of immunostimulatory molecules, tumor antigens, RNA-based therapeutics, or anticancer drugs [14,19,20]. Together, these mechanisms highlight the dual role of EVs as both biomarkers of disease status and active mediators of pathogenesis or therapeutic response, an important consideration for interpreting veterinary biomarker studies and evaluating the safety and efficacy of EV-based interventions.

Table 1: *Distinctive Properties of Extracellular Vesicle Subgroups (47).*

Specifications	***Exosome***	***Microvesicle***	***Apoptotic bodies***
Size	*30–150 nm*	*Up to 1000 nm*	*Up to 5000 nm*
Intracellular origin	*Endosomal pathways*	*Plasma membrane*	*Plasma membrane; during apoptosis*
Sucrose gradient	*1.12–1.19 g/mL*	*1.04–1.07 g/mL*	*1.16–1.28 g/mL*
Appearance in TEM	*Round shaped*	*Heterogeneous*	*Heterogeneous*
Markers	*Tetraspanins (CD9, CD63, CD81, CD82), TSG101, Alix, Flotillin, Heat Shock Proteins (Hspa8, Hspa60, Hspa70, Hspa90), ESCRT components, MHC-I and MHC-II molecules*	*Non-specific markers; Integrins, selectins, CD40, and Annexin V-positivity; ARF6, VCAMP3*	*High PS level, TSP, C3b, Annexin V-positivity*
Internal contents	*mRNA, miRNA, ncRNA, tRNA, siRNA and rarely DNA; Cytoplasmic and membrane proteins (actin, myosin, syntenin, etc.); Lipids (phosphoglycerides, cholesterol, ceramide, sphingomyelin, fatty acid chains); Carrier proteins (e.g., albumin); Metabolic enzymes (GAPDH, LDHA, PGK1, PKM, PK, ATPase, enolase, aldolase)*	*mRNA, miRNA, ncRNA, cytoplasmic and membrane proteins, and thermal shock proteins*	*Cellular organelles and nuclear fragments*
Detection method	*Flow-cytometry, electron microscopy, nanoparticle tracking analysis (NTA), Western blotting assay*	*Flow-cytometry and electron microscopy*	*Flow-cytometry and electron microscopy*

3. ISOLATION, PURIFICATION, and CHARACTERIZATION METHODOLOGIES of EVs

In extracellular vesicle (EV) research, pre-analytical variables are among the most important determinants of data quality, reproducibility, and biological interpretability. Their impact may be particularly substantial in biomarker discovery and functional studies, where uncontrolled variation in sample collection, processing, and storage can obscure or even exceed true disease-related biological signals [2,3]. This issue is especially relevant in veterinary medicine because EV profiles are influenced not only by disease status, but also by species, breed, age, sex, diet, housing conditions, season, lactation stage, estrous cycle, stress, infection, and drug exposure [1,4,5]. Therefore, EV studies should include rigorous standardization and transparent reporting of pre-analytical conditions. Essential information includes animal metadata, physiological status, disease definition and severity, timing of sample collection relative to feeding, exercise, or treatment, sampling method, anticoagulant type for blood-derived samples, time to processing, centrifugation and filtration procedures, storage temperature and duration, and the number of freeze–thaw cycles [2,3,5]. In functional assays, additional details such as EV dose normalization, use of heat-inactivated preparations, and appropriate negative or vehicle controls should also be reported [2,3,21].

Matrix-specific confounders must also be carefully considered. Milk and colostrum contain caseins, fat globules, and protein aggregates that may co-isolate with EVs and interfere with particle counting, proteomic analysis, and transcriptomic profiling [1,8,9]. Plasma and serum are rich in lipoproteins and soluble proteins that overlap with EVs in size and density, increasing the risk of contamination, especially when high-yield but low-specificity isolation methods are used [3,8]. Urine generally contains fewer proteins, but urinary EVs are strongly affected by hydration status, collection method, cellular contamination, and protease activity [5,22]. Similarly, semen and reproductive tract fluids contain complex protein and lipid backgrounds, and their EV composition may vary according to sexual maturity, season, and collection frequency [16,23].

Taken together, careful control of pre-analytical variables is not merely a technical requirement but a central condition for reliable EV research. Without standardized sampling, processing, storage, and reporting, comparisons between studies, species, disease states, or therapeutic interventions remain difficult and potentially misleading.

3.1 Isolation methods of EVs

EV extraction and enrichment can be performed using several methodological approaches, each with specific advantages and limitations. Common methods include differential ultracentrifugation, density-gradient ultracentrifugation, size-exclusion chromatography, ultrafiltration, polymer-based precipitation, immunoaffinity capture, microfluidic isolation platforms, and commercial EV isolation kits. Because no single method can isolate completely pure EV populations, the choice of technique should be guided by the sample type, study objective, required purity, downstream application, and need for scalability or clinical translation.

3.1.1 Differential ultracentrifugation

Differential ultracentrifugation (dUC) remains one of the most widely used approaches for EVs enrichment because of its conceptual simplicity and broad accessibility in academic laboratory settings [1,19]. In a conventional workflow, sequential low-speed centrifugation steps are first used to remove intact cells and larger debris, followed by intermediate-speed spins to sediment larger vesicles and apoptotic bodies, and finally high-speed ultracentrifugation—often around 100,000 g—to recover fractions enriched in small EVs [19,20]. Despite its widespread use, dUC should be considered an enrichment strategy rather than a true purification method. In practice, it frequently co-pellets protein aggregates, lipoproteins, and other nanoscale contaminants, and under harsh or repeated conditions it may also promote vesicle aggregation or structural damage [3,8,20].

The enduring popularity of dUC is largely attributable to its operational familiarity, its capacity to process moderate sample volumes, and its independence from specialized proprietary consumables. Nevertheless, its limitations are substantial. Purity can vary markedly across laboratories and sample types, and dUC alone is often insufficient for high-confidence biomarker discovery or mechanistic interpretation unless followed by an additional cleanup step such as size-exclusion chromatography or density gradient separation [3,8,20]. The method is also time-intensive and highly sensitive to rotor characteristics, tube geometry, sample viscosity, and operator-dependent procedural differences. In veterinary milk and colostrum studies, these concerns are magnified because dUC without appropriate defatting and casein management may lead to substantial co-isolation of non-EV materials [1,9]. Consequently, dUC-derived preparations are most appropriately described as ultracentrifugation-enriched EVs rather than definitive exosome isolates unless further evidence supports a narrower subtype claim [2,3].

3.1.2 Density gradient ultracentrifugation

Density gradient ultracentrifugation, typically using sucrose or iodixanol gradients, separates particles according to buoyant density and is generally regarded as a higher-purity alternative to dUC alone [19,20]. In veterinary biomarker studies, this added selectivity can be particularly valuable because it helps distinguish EV-associated signals from co-isolated lipoproteins and soluble protein complexes, thereby improving confidence in both omics-based analyses and functional assays [3,8,20]. At the same time, the method is laborious, relatively low-throughput, and often associated with reduced overall yield, which limits its practicality for large-scale clinical workflows. Moreover, although density gradients improve purity, they do not provide absolute selectivity because some non-EV particles share overlapping density ranges with vesicles [3,8]. Thus, density-based separation is best viewed as a high-rigor option for mechanistic or discovery-oriented studies in which minimizing non-EV confounding is more important than maximizing processing speed.

3.1.3 Size-based isolation methods: filtration, ultrafiltration, and size-exclusion chromatography

Size-based approaches occupy an important middle ground between accessibility, scalability, and preservation of EV integrity. Filtration and ultrafiltration use membrane pore sizes or molecular weight cutoffs to remove larger contaminants and concentrate EV-containing fractions [20]. These methods are often useful as upstream processing steps, such as 0.22 μm filtration to reduce larger particles before downstream purification. However, aggressive filtration may deform vesicles, promote membrane adsorption, or selectively bias recovery toward more mechanically rigid particles [20,21]. In complex and viscous veterinary matrices such as milk or semen, membrane clogging and inconsistent recovery remain practical limitations. Tangential

flow filtration (TFF) has become increasingly attractive for translational applications because it enables processing of larger sample volumes with improved scalability and gentler fluid dynamics than conventional dead-end ultrafiltration [20,21]. This feature makes TFF particularly relevant for therapeutic EV manufacturing, including mesenchymal stromal cell-derived EV production, where reproducible yield and compatibility with semi-closed or closed systems are important. Nevertheless, TFF should not be interpreted as a stand-alone purity solution, since downstream polishing—often by SEC—may still be required depending on the source matrix and intended application [21].

Among size-based methods, size-exclusion chromatography (SEC) is widely valued because it can preserve vesicle integrity while reducing contamination from soluble proteins [20]. In SEC, EV-sized particles typically elute earlier than smaller proteins due to differential entry into porous beads. For veterinary biomarker studies, this characteristic makes SEC especially useful when sample volumes are moderate and downstream analyses such as proteomics or functional assays are sensitive to protein carryover [3,20]. However, SEC is not fully selective, as similarly sized non-EV particles, including certain lipoproteins, may still co-elute [3,8,20]. In addition, the method often dilutes the recovered sample, necessitating a subsequent concentration step that may reintroduce losses or recovery bias.

3.1.4 Polymer-based precipitation

Polymer-based precipitation methods remain popular because they are rapid, inexpensive, and technically simple, requiring little specialized equipment [20]. Their principal advantage is the ability to recover relatively high particle and protein yields, which may appear attractive in exploratory or resource-limited settings. However, the recovered material often contains substantial amounts of non-EV contaminants, including protein aggregates and lipoproteins, which can confound omics analyses and exaggerate apparent biological activity in functional assays [3,8,20]. For this reason, precipitation-based workflows may have utility in preliminary screening or in settings with limited infrastructure, but their outputs should be interpreted with caution and, whenever possible, validated against higher-purity isolation strategies.

3.1.5 Immunoaffinity capture

Immunoaffinity-based methods isolate EVs through antibodies directed against surface-associated proteins, such as tetraspanins, or against disease-relevant antigens [20]. In theory, this can increase specificity and enable selective enrichment of biologically relevant EV subsets, including tumor-associated vesicle populations. In practice, however, immunocapture introduces an inherent selection bias because only vesicles expressing the chosen target are recovered. This issue is particularly relevant in veterinary medicine, where antibody cross-reactivity is not always well established and EV marker expression may differ across species [1,5,20]. Additional limitations include higher cost, limited scalability, and reduced suitability for large cohort studies or therapeutic manufacturing. As a result, immunoaffinity approaches are best considered targeted analytical tools rather than universal isolation solutions.

3.1.6 Microfluidics and emerging technologies

Microfluidic technologies are evolving rapidly as platforms for EV isolation and integrated analysis, exploiting principles such as size discrimination, immunoaffinity, acoustofluidics, dielectrophoresis, and on-chip detection [21,24]. Their major appeal in veterinary medicine lies in the possibility of reducing workflow complexity, lowering sample volume requirements, and ultimately moving toward point-of-care or near-patient testing. Such systems may be especially promising for herd-level surveillance applications, such as mastitis screening, or for rapid monitoring in companion animal oncology. Nevertheless, important barriers remain, including device standardization, susceptibility to clogging when complex fluids such as milk or whole blood are used, and the need for rigorous benchmarking against established reference methods [21,24]. Their future impact in veterinary practice will therefore depend not only on technical performance but also on demonstration of robustness, affordability, and clinical utility.

Table 2: *Comparison of Common Extracellular Vesicle (EV) Isolation Methods*			
Isolation Method	***Mechanism***	***Advantages***	***Disadvantages***
Differential Centrifugation	*Sequential centrifugation at increasing speeds to remove cellular debris and large vesicles, eventually pelleting exosomes at high speeds.*	*Considered a standard and highly common gold-standard approach for isolation from various biofluids and media.*	*Low efficiency when processing viscous biological fluids such as plasma and serum.*
Density Gradient Centrifugation	*Combines ultracentrifugation with a density gradient medium (e.g., sucrose or iodixanol/OptiPrep) to separate particles based on buoyant density.*	*Allows for high-purity separation of exosomes from other vesicles, fragments, and contaminants of different densities.*	*Highly sensitive to centrifugation time; requires specialized technical expertise.*
Size-Exclusion Chromatography (SEC)	*Separation of macromolecules based on size as they pass through a column packed with porous polymer beads.*	*Enables precise separation of large and small molecules. Unlike centrifugation, EV structure is preserved from shear stress.*	*Time-consuming process, which limits the throughput when processing multiple biological samples simultaneously.*
Ultrafiltration	*Uses semi-permeable membranes with specific pore sizes to capture exosomes while allowing smaller proteins and solutes to pass.*	*Effective for concentrating EVs and separating them from small soluble molecules and debris.*	*Potential for EVs to adhere to the membrane (loss of yield); pressure-induced deformation or damage to EVs may occur.*
Polymer-based Precipitation	*Mixing biofluids with a polymer solution (e.g., PEG) followed by incubation and low-speed centrifugation to precipitate EVs.*	*Minimal impact on the biological integrity of EVs; operates at neutral pH and is relatively simple to perform.*	*High risk of co-isolating non-vesicular contaminants (e.g., lipoproteins). Polymer residues may interfere with downstream analyses.*
Immunological Methods	*Targeted capture using antibody-coated magnetic beads or advanced ELISA-based platforms to bind specific surface markers.*	*Enables highly specific isolation of total EVs or selective subpopulations. Can be used for protein quantification and identification.*	*Not suitable for large sample volumes; potential loss of biological function of the isolated vesicles due to antibody binding.*

3.2 Characterization of EVs

Because no currently available isolation method can generate completely pure extracellular vesicle (EV) preparations, EV characterization should be based on multiple complementary, or orthogonal, analytical approaches. This strategy is essential to confirm that the recovered material is consistent with EVs while also identifying potential contaminants such as protein aggregates, lipoproteins, cell debris, fat globules, or other non-vesicular particles [2,3]. In veterinary EV research, particularly in biomarker discovery and therapeutic studies, particle analysis, morphological evaluation, and molecular marker assessment should therefore be integrated rather than interpreted separately.

Particle sizing and concentration are commonly assessed using nanoparticle tracking analysis (NTA) and tunable resistive pulse sensing (TRPS) [3,20]. These methods are useful for estimating particle size distribution, comparing EV batches, and calculating doses for functional experiments. However, they cannot confirm vesicular identity or distinguish EVs from similarly sized contaminants. Therefore, an increase in particle number should not be interpreted as a true increase in EV abundance unless supported by additional evidence of EV enrichment, purity, and molecular composition [3,8]. Detailed reporting of instrument settings,

calibration procedures, dilution factors, sample viscosity correction, and data acquisition parameters is essential, especially when working with complex veterinary fluids.

Morphological assessment using transmission electron microscopy (TEM) or cryo-electron microscopy (cryo-EM) provides visual evidence of vesicle-like structures and can help identify non-vesicular contaminants [3,20]. This is particularly valuable in complex matrices such as milk and colostrum, where fat globules, casein micelles, and protein aggregates may interfere with EV interpretation [9]. However, imaging findings should be interpreted alongside biochemical and particle-based analyses, as sample preparation may introduce artifacts.

Molecular characterization is another central component of EV validation. Western blotting, ELISA, bead-based assays, or other immunoassays are commonly used to detect EV-associated proteins such as tetraspanins, endosomal markers, or cell-type-specific molecules [2,3]. In veterinary species, however, marker selection requires particular caution because antibody specificity, cross-reactivity, and antigen conservation are not always validated across species. Consequently, limited marker panels should not be used to make strong claims about EV subtype identity. A more rigorous approach is to report the markers tested, specify antibody validation for the target species, include appropriate positive and negative controls, and interpret results as evidence of EV enrichment rather than definitive proof of a specific EV subtype [2,3,5].

Advanced analytical platforms, including high-sensitivity flow cytometry, imaging flow cytometry, and single-particle analysis technologies, can further reveal EV heterogeneity and subpopulation structure [21]. In addition, omics-based approaches, including proteomics, transcriptomic, small RNA sequencing, and lipidomic, can identify candidate biomarkers and mechanistic pathways. Nevertheless, these techniques are highly sensitive to contamination by abundant non-EV molecules, particularly lipoproteins, soluble proteins, protein aggregates, and matrix-specific contaminants [3,8]. Therefore, omics studies should be supported by rigorous sample preparation, contamination assessment, technical replicates, batch-correction strategies, and validation in independent cohorts.

Overall, reliable EV characterization in veterinary research requires a multi-method validation framework that combines particle analysis, morphology, molecular markers, and, when appropriate, single-particle or omics-based profiling. This integrated approach improves reproducibility, reduces the risk of over interpretation, and strengthens the translational value of EV-based biomarkers and therapeutics.

4. DIAGNOSTIC and BIOMARKER APPLICATIONS of EVs in VETERINARY MEDICINE

EVs have emerged as versatile biological entities with considerable promise in both diagnostics and therapeutics in veterinary medicine. Owing to their lipid bilayer structure and cell-specific molecular cargo, EVs carry proteins, lipids, metabolites, and regulatory nucleic acids that reflect the physiological state of their cells of origin. As such, they function as minimally invasive, biologically informative readouts of tissue homeostasis, inflammation, infection, reproductive status, and neoplastic transformation. Their accessibility in a range of veterinary biofluids, including blood, milk, urine, semen, follicular fluid, uterine secretions, and saliva, positions them as attractive candidates for liquid biopsy applications across companion animal, equine, and livestock medicine [16,47]. The diagnostic value of EVs is particularly compelling in veterinary practice, where repeated tissue sampling is often impractical, herd-level surveillance may be required, and early disease detection can substantially influence both animal welfare and economic productivity. In parallel, EVs are gaining increasing attention as therapeutic agents, particularly in regenerative and immunomodulatory contexts. In many settings, they may recapitulate key paracrine effects of parental cells, especially mesenchymal stromal/stem cells (MSCs), while offering potential advantages in stability, storage, and manufacturability. However, the translational promise of EVs must be interpreted with caution. Their clinical adoption will depend on rigorous standardization, orthogonal characterization, dose definition, potency assessment, and evidence of clinical utility in relevant veterinary populations [21,25,30–33].

4.1 Diagnostic Applications

One of the most promising applications of EVs in veterinary medicine is their use as liquid biopsy platforms. Because EVs encapsulate disease-associated molecular signatures, they can provide insight into ongoing cellular and tissue processes without the need for invasive sampling. This is particularly valuable in veterinary species, where access to affected tissues may be limited and serial monitoring is often necessary. EV-based diagnostics may therefore support earlier disease detection, phenotype stratification, prognosis, treatment monitoring, and longitudinal assessment of therapeutic response [4, 6].

Compared to conventional circulating biomarkers, EVs provide superior diagnostic potential due to their lipid-protected cargo, which reflects active cellular signaling pathways rather than passive tissue leakage.

Furthermore, their multimodal composition enables comprehensive molecular profiling. However, the translation of EV-based diagnostics into clinical practice necessitates addressing significant methodological challenges, including pre-analytical variability, sample heterogeneity, and the presence of matrix-specific contaminants [4, 5, 6].

4.1.1 Bovine Mastitis and Dairy Health

Bovine mastitis remains one of the most important inflammatory diseases in dairy production, with major consequences for milk yield, quality, animal welfare, antimicrobial use, and culling rates. Conventional diagnostic approaches, such as somatic cell count, microbiological culture, and clinical scoring, remain central to disease management but have recognized limitations in sensitivity, specificity, and early detection. EVs present an attractive complementary approach because milk is a readily accessible biofluids that can be sampled repeatedly and non-invasively [4,12,13].

Milk- and colostrum-derived EVs are especially intriguing in veterinary medicine because they offer a naturally abundant and potentially scalable source of biologically active vesicles. Milk-derived EVs originate primarily from mammary epithelial cells and immune cells and may carry molecular signatures associated with inflammation, epithelial stress, pathogen exposure, and tissue remodeling. These preparations may be particularly attractive for immunomodulatory, intestinal, neonatal, and mucosal applications. Their accessibility could make them more economically feasible than many engineered or culture-derived EV products, especially in livestock systems. However, milk-based EVs isolation and therapeutics also pose substantial challenges. The composition of milk EVs varies with lactation stage, species, diet, health status, and processing conditions. In addition, milk is a complex matrix containing proteins, lipids, and particles that can confound isolation and downstream interpretation. For these reasons, rigorous purification, quality control, and product characterization are essential before clinical translation can be considered. Cross-species use adds another layer of complexity and should not be assumed to be interchangeable without evidence of safety and efficacy [12,40–42].

Somatic cell count (SCC) and bacterial culture remain foundational tools for mastitis diagnosis, but each has clear constraints: SCC is sensitive yet limited for etiologic stratification, whereas culture may be time-consuming and yield false-negative results, particularly in intermittent shedding or after antibiotic exposure [4,10]. EV-derived biomarkers offer a complementary strategy by capturing host-response signatures that may emerge earlier than SCC, distinguish inflammatory endotypes, and reflect resolution versus persistence during or after treatment [12,13]. EV-associated miRNAs implicated in NF-κB signaling, cytokine regulation, epithelial barrier function, and leukocyte recruitment are plausible candidates because they align mechanistically with core mastitis immunopathology [12,13]. Likewise, milk EV proteomic patterns may report epithelial injury, innate immune activation, and oxidative stress responses, supporting the rationale for multi-analyte panels rather than reliance on single markers.

In particular, EV-associated microRNAs and proteins involved in innate immune signaling, cytokine regulation, and barrier integrity have emerged as potential biomarkers for mastitis detection and stratification. These markers may be especially valuable for identifying subclinical disease, monitoring progression, and distinguishing resolving from persistent inflammation. However, the diagnostic translation of milk EVs will require careful control of confounding factors such as lactation stage, parity, diet, milking interval, and sample handling, all of which can influence EV abundance and cargo composition [41-43].

4.1.2 Reproductive Health and Fertility

EVs also play important roles in reproductive biology and may serve as informative biomarkers of fertility and reproductive competence. They have been identified in seminal plasma, follicular fluid, oviductal fluid, uterine secretions, and embryo culture media, where they participate in gamete maturation, fertilization, embryo development, and maternal–embryo communication. Because these processes depend on tightly coordinated intercellular signaling, EV cargo may reflect reproductive tract health, hormonal milieu, inflammatory status, and developmental competence [13, 47, 18].

In males, seminal plasma EVs have been linked to sperm motility, capacitation, membrane stability, and fertilization potential. In female reproductive systems, EVs may contribute to follicular development, endometrial receptivity, embryo implantation, and regulation of local immune tolerance. These features make EVs promising candidates for fertility biomarker development in both livestock and companion animals. Nonetheless, reproductive EV studies are inherently sensitive to biological variation driven by estrous cycle stage, breed, age, season, and reproductive management practices. Therefore, successful translational

application will require robust cohort stratification and validation in well-defined clinical populations [23,26,27].

4.1.3 Infectious Diseases and Host–Pathogen Interactions

Veterinary infectious diseases, including bacterial, viral, and parasitic conditions, also represent a promising area for EV-based diagnostics. Both host-derived and pathogen-associated EVs can influence immune responses, inflammation, and intercellular communication during infection. Pathogens can hijack EV pathways to facilitate their entry into host cells or to modulate the host immune system. As a result, EV cargo may capture early host-response signatures before overt clinical signs develop. This feature is particularly relevant in diseases where pathogen detection is difficult or intermittent, or where inflammatory changes precede conventional diagnostic abnormalities. Conversely, host-derived EVs can act as antiviral or antibacterial agents by delivering microRNAs or other effectors [29-32]. Understanding these intricate interactions through EV-based research could lead to novel diagnostic tools for pathogen detection and innovative therapeutic strategies to combat infectious agents in veterinary patients. In infectious disease settings, EV-based assays may offer advantages over single-analyte approaches by integrating information from multiple biological pathways. However, a major challenge lies in distinguishing infection-specific EV signatures from generalized inflammatory responses. Co-infections, concurrent disease, stress, and environmental variation may further complicate biomarker interpretation under field conditions. Accordingly, clinically useful EV diagnostics will likely require multi-marker panels, well-defined endpoints, and validation in naturally occurring disease populations rather than only in experimental challenge models [10, 24, 28].

4.1.4 Animal Oncology

Cancer in dogs and cats is a major clinical challenge and an important domain of comparative oncology. Tumor-derived EVs, as well as host EVs shaped by the tumor microenvironment, can carry oncogenic proteins, regulatory RNAs, lipids, and immunomodulatory molecules that reflect tumor burden, metastatic potential, and treatment response. These properties make EVs attractive candidates for minimally invasive cancer diagnostics and monitoring. Current findings suggest that circulating EV profiles differ between healthy and cancer-bearing animals, supporting their potential utility as biomarkers for detection, staging, prognosis, and relapse surveillance. However, the field remains in an early translational phase, with many studies limited by small sample sizes, inconsistent isolation methods, and incomplete clinical validation. The greatest value of EVs in veterinary oncology may ultimately lie in multi-analyte, disease-specific panels integrated with clinical staging and imaging rather than in single universal cancer markers [16,29].

4.2 Therapeutic Applications

4.2.1 EVs as Cell-Free Therapeutics

Beyond diagnostics, EVs are increasingly being explored as therapeutic agents in veterinary medicine. This interest is driven largely by the recognition that many beneficial effects of cell-based therapies, particularly those involving MSCs, are mediated by paracrine signaling rather than direct engraftment. EVs may therefore serve as a cell-free platform capable of delivering anti-inflammatory, immunomodulatory, pro-angiogenic, anti-apoptotic, and pro-regenerative signals while potentially avoiding some of the limitations associated with cells administration [21,25,30–33]. Compared with whole-cell products, EVs may offer advantages in terms of scalability, storage, sterilization, and repeat dosing. Their nanoscale size may also enhance tissue penetration and reduce certain safety concerns related to cell entrapment or uncontrolled proliferation. Nevertheless, EVs are not inherently simpler to develop or standardize. Their biological activity depends strongly on cellular source, isolation method, purification strategy, cargo composition, and product handling. For clinical translation, EV therapeutics must therefore be treated as rigorously defined biologics rather than as generic cell-free extracts [21,25,34].

4.2.2 MSC-Derived EVs in Regenerative Medicine

Among EV-based therapeutic platforms, MSC-derived EVs are the most extensively studied in veterinary medicine. MSCs from bone marrow, adipose tissue, umbilical cord, amniotic tissue, and synovium have already been investigated in a range of species and clinical settings, and EVs represent a logical extension of this work. MSC-EVs are thought to exert therapeutic effects by modulating inflammation, shaping macrophage polarization, reducing apoptosis, supporting angiogenesis, and promoting matrix remodeling and tissue repair. These properties make MSC-EVs especially attractive for disorders with a strong inflammatory and

degenerative component, including osteoarthritis, tendon and ligament injuries, chronic wounds, and inflammatory soft tissue lesions. In equine medicine, they may be particularly relevant for musculoskeletal repair and performance recovery, while in canine medicine they could complement existing regenerative approaches for joint disease and wound healing. Nevertheless, the field still requires stronger evidence regarding biodistribution, retention time, dose–response relationships, and long-term structural outcomes. At present, many studies demonstrate encouraging biological effects, but fewer establish durable clinical benefit [1,21,25,35, 36].

4.2.3 Orthopedic Applications

Orthopedic disease is among the most advanced and clinically relevant indications for EV therapy in veterinary medicine. Osteoarthritis, tendon injury, and ligament damage are highly prevalent in horses and dogs and represent major causes of pain, reduced performance, and economic loss. EVs may help attenuate synovial inflammation, support chondrocyte survival, and preserve extracellular matrix integrity, thereby reducing the downstream consequences of tissue injury. A key advantage of EVs in orthopedic applications is their potential for localized administration, including intra-articular delivery, which may improve target exposure while limiting systemic effects. However, orthopedic success will depend not only on symptom improvement but also on evidence of structural preservation and functional durability. Imaging, gait analysis, biomarker studies, and long-term follow-up will therefore be essential to move this field from experimental promise to routine veterinary practice [37–41].

4.2.4. Wound Healing

Wound healing is another highly promising application of EVs in veterinary medicine. Successful repair requires coordinated regulation of inflammation, angiogenesis, fibroblast activity, extracellular matrix deposition, and re-epithelialization, all of which may be influenced by EV cargo. MSC-derived EVs have been associated with enhanced cell migration, improved angiogenic signaling, reduced inflammatory burden, and accelerated tissue repair in preclinical studies. These properties are of particular interest in species and clinical contexts where wound healing is challenging, such as distal limb wounds in horses or chronic skin lesions in companion animals. EV-based approaches may also be combined with hydrogels, scaffolds, or topical formulations to improve retention and local bioavailability. In this context, the delivery system is often inseparable from the therapeutic effect, and future studies should explicitly define the contribution of both the EV product and the carrier platform.

4.2.5. Female Reproductive Therapeutics

The reproductive tract represents an emerging therapeutic target for EVs because EV-mediated communication is already integral to reproductive physiology. In theory, exogenous EVs could modulate uterine inflammation, support endometrial repair, improve embryo culture systems, and enhance reproductive tract homeostasis after infectious or inflammatory insult. Such applications are particularly attractive in livestock medicine, where even modest improvements in fertility can have substantial economic consequences. Despite this promise, reproductive EV therapies remain at an early stage. Their success will likely depend on precise timing, dose, route of administration, and source selection, as well as on the reproductive status of the recipient. As in other areas of EV therapeutics, mechanistic plausibility should not be conflated with clinical readiness [47].

4.2.6 Colostrum- and milk-derived EVs: special considerations and advantages

Colostrum and early-lactation milk contain abundant EVs enriched in immunoregulatory cargo with potential relevance to neonatal immune development and gastrointestinal maturation, but these same features can be leveraged diagnostically because inflammatory perturbations in the mammary gland are likely to be strongly reflected in early secretions [12]. Translationally, two closely related directions emerge. First, milk EV profiles could support earlier detection, risk stratification, or longitudinal monitoring of mastitis and related mammary health conditions. Second, colostrum-derived EVs are being explored as bioactive agents that may modulate inflammation and promote tissue repair, suggesting an intervention logic that could ultimately complement mastitis mitigation strategies [12]. For the diagnostic pathway, however, strict control and reporting of lactation stage, parity, milking interval, and storage conditions are indispensable because these factors can substantially shift EV abundance and cargo composition independent of disease status [5,12].

4.2.7 Immunomodulation and Host-Response Reprogramming

A recurring theme across EV therapeutic studies is the ability of EVs to reshape inflammatory and immune responses rather than directly replace damaged tissue. This is particularly relevant in veterinary diseases where inflammation drives pathology, including osteoarthritis, mastitis-associated tissue injury, inflammatory skin disease, and postoperative healing responses. MSC-EVs may promote resolution of inflammation by influencing macrophage phenotype, reducing pro-inflammatory cytokine production, and supporting pro-repair signaling networks. This immunomodulatory capacity is clinically meaningful, but it must be interpreted appropriately. Improvement in pain, swelling, or inflammatory markers does not necessarily imply complete tissue regeneration. Accordingly, therapeutic studies should distinguish between anti-inflammatory efficacy and true structural restoration, and outcome measures should be aligned with the intended clinical use [30–33,37,43].

4.2.8 EVs in Oncology and cancer immunotherapy

EVs also hold potential in veterinary oncology, both as delivery vehicles and as immunomodulatory platforms. Engineered or selected EVs may be used to transport chemotherapeutic drugs, tumor antigens, nucleic acids, or immune-stimulatory molecules, suggesting possible roles in cancer vaccination, targeted therapy, and combination treatment strategies. Companion animals, especially dogs with spontaneous tumors, provide an important translational bridge for this work. At the same time, oncology is a particularly complex EV application because tumor-derived EVs can themselves promote immune evasion, angiogenesis, and metastatic spread. This dual biological role means that safety, source selection, and mechanistic validation are especially critical. For now, oncology remains a promising but highly cautious frontier for EV-based therapeutics in veterinary medicine [14,44,45].

4.3 EVs as Drug Delivery Systems and Engineered Biologics

Extracellular vesicles (EVs) represent a promising frontier in veterinary medicine, particularly for their potential as therapeutic agents and sophisticated drug delivery vehicles. Their endogenous nature allows them to navigate biological barriers and deliver payloads, including nucleic acids, proteins, and small molecules, to target cells [1, 2]. The cargo inherent to EVs can also be manipulated, enabling the engineering of EVs with specific therapeutic functions [3]. This capacity extends to the development of engineered biologics, where EVs can be modified to express therapeutic proteins or deliver genetic material for gene therapy applications [4, 5]. The inherent biocompatibility and low immunogenicity of EVs make them attractive alternatives to synthetic delivery systems, offering a natural platform for enhanced drug targeting and reduced systemic toxicity [6, 7].

5. TRANSLATIONAL CONSIDERATIONS and FUTURE DIRECTIONS

The translation of EV-based therapies from preclinical research to clinical application in veterinary medicine presents a complex but achievable goal. Standardization of EV isolation, characterization, and functional assessment is paramount for ensuring reproducibility and therapeutic efficacy [8]. Challenges include scaling up production to meet clinical demands, establishing robust quality control measures, and navigating the regulatory landscape for these novel biologics [9]. Future research should focus on developing standardized protocols for EV manufacturing, preclinical validation in relevant animal models, and pilot clinical trials to assess safety and efficacy [10]. Addressing these translational hurdles will pave the way for the routine clinical use of EV therapeutics in veterinary practice.

5.1 Biological Heterogeneity and Context Dependence

A significant consideration in the application of EVs is their inherent biological heterogeneity. EVs encompass a diverse range of subpopulations (e.g., exosomes, microvesicles, apoptotic bodies) with varying sizes, compositions, and origins [11, 4]. This heterogeneity can impact their biological activity and therapeutic potential, necessitating precise characterization methods for different EV types [12]. Furthermore, the biological context in which EVs are produced and function is crucial. Factors such as the source cell type, physiological state of the donor animal, and the microenvironment of recipient cells can significantly influence EV cargo and function [13, 14, 5]. Understanding and controlling this context-dependent behavior is essential for harnessing the full therapeutic promise of EVs.

5.2 Terminology and Operational Definitions

The field of EV research is challenged by inconsistent terminology and a lack of universally accepted operational definitions. This ambiguity can hinder inter-laboratory comparisons and impede the progress of

clinical translation. For instance, the distinction between exosomes and other EV subtypes is often based on size, which can be variable and overlapping. Establishing standardized nomenclature and precise definitions for EV subtypes and their associated markers is critical for clear communication and the advancement of the field. Such standardization will facilitate the interpretation of research findings and the development of reliable diagnostic and therapeutic strategies [2,3].

5.3 Isolation, Contamination, and Matrix Effects

The isolation of EVs from biological fluids is a critical step that can significantly influence downstream analysis and therapeutic applications. Various isolation methods exist, each with its own advantages and limitations regarding purity, yield, and recovery of specific EV subpopulations. Common challenges include contamination with non-vesicular biomolecules (e.g., proteins, nucleic acids) and other cellular debris, which can confound experimental results. In milk, casein micelles, fat globule components, and processing-derived aggregates can similarly bias particle counting, proteomics, and functional readouts [1,9,12]. In reproductive fluids, viscosity and high protein backgrounds complicate recovery and can create selective loss of specific EV subpopulations [16,23]. Furthermore, the presence of the biological matrix (e.g., serum, plasma, urine) can interfere with EV isolation and characterization, leading to potential artifacts and inaccurate assessments. Developing robust, reproducible, and standardized isolation protocols that minimize contamination and matrix effects is essential for reliable EV research and application [3, 8, 20].

5.4 Study Design Standards for EV-Based Biomarkers

The development of EV-based biomarkers for disease diagnosis and monitoring in veterinary medicine requires rigorous study design and validation. Standardized methodologies for EV isolation, quantification, and characterization are crucial to ensure the reliability and reproducibility of biomarker discovery. This includes defining precise analytical techniques for measuring EV concentration, size distribution, and the presence of specific molecular markers. Furthermore, robust statistical analysis and validation in large, diverse cohorts of animals are necessary to confirm the diagnostic accuracy and prognostic value of proposed EV biomarkers. Establishing such standards will accelerate the translation of EV biomarkers from the research laboratory to clinical utility [19, 20, 21].

5.5 Livestock Applications

EVs hold significant promise for improving the health and productivity of livestock. In livestock systems, EV-based approaches are particularly relevant to diseases and physiological processes with major economic consequences. Bovine mastitis, for example, remains one of the most prevalent and costly inflammatory disorders in dairy production, reducing milk yield and quality while increasing treatment costs and culling rates [10,11]. Conventional diagnosis often relies on somatic cell counts, microbiological culture, or clinical signs, but these methods may not fully capture early molecular changes or distinguish disease subtypes with sufficient precision. EVs derived from milk, mammary epithelial cells, and immune cells have therefore attracted considerable interest as reservoirs of proteins, microRNAs, and inflammatory mediators that may enable earlier detection, improved disease stratification, and potentially even therapeutic intervention [10,12]. Similarly, in reproductive medicine, EVs have emerged as central mediators of communication within the ovarian follicle, oviduct, uterus, placenta, and male reproductive tract, where they influence oocyte maturation, sperm capacitation, fertilization, embryonic development, implantation, and maternal-fetal signaling [13–15]. These functions make them highly relevant not only to fertility management in cattle, buffalo, swine, and equine species, but also to assisted reproductive technologies and embryo culture systems.

In livestock medicine, EV-based research is increasingly focused on conditions with significant economic impact, such as bovine mastitis. While conventional diagnostics—including somatic cell counts and microbiological cultures—often lack the sensitivity to capture early molecular pathology or disease subtyping, milk-derived EVs provide a rich, non-invasive source of biomarkers, including microRNAs and inflammatory mediators. Furthermore, in the field of reproductive biology, EVs serve as critical signaling hubs within the reproductive tract. By regulating essential processes from gamete maturation to maternal-fetal communication, EVs offer transformative potential for fertility management and the optimization of assisted reproductive technologies across major livestock species, including cattle, swine, and equines

In companion animals, EV research is gaining momentum in oncology, orthopedics, dermatology, neurology, and chronic inflammatory disease [8,16]. Canine and feline cancers, including lymphoma, mammary tumors, osteosarcoma, and melanoma, frequently serve as both veterinary clinical challenges and comparative models for human disease. Tumor-derived EVs are increasingly implicated in cancer progression

through the modulation of angiogenesis, extracellular matrix remodeling, immune evasion, premetastatic niche formation, and drug resistance [16,17]. At the same time, their molecular contents provide an opportunity for minimally invasive biomarker development, while engineered or cell-derived EVs are being explored as therapeutic platforms for anticancer vaccination, targeted drug delivery, and immune reprogramming [8,17]. Research into EV-based strategies for disease prevention, early detection, and targeted treatment in livestock populations is a burgeoning area with substantial economic and animal welfare implications.

5.6 Future Directions: Precision Veterinary Medicine and One Health

The future of EVs in veterinary medicine lies in their integration into precision veterinary medicine and the broader One Health initiative. By leveraging the ability of EVs to carry specific molecular signatures, tailored diagnostic and therapeutic approaches can be developed for individual animals based on their unique genetic makeup, health status, and environmental exposures [35]. This personalized approach can optimize treatment efficacy and minimize adverse events. Furthermore, EVs can serve as crucial communicators in the One Health paradigm, bridging the gap between human, animal, and environmental health by facilitating the understanding and management of zoonotic diseases and shared environmental health challenges [37].

6. CONCLUSION

EVs represent a transformative platform with immense potential for revolutionizing veterinary diagnostics and therapeutics. Their inherent capacity for intercellular communication, cargo delivery, and engineered modification positions them as versatile tools for addressing a wide spectrum of animal health challenges, from infectious diseases and cancer to reproductive disorders. While significant progress has been made, continued research focused on standardization, robust validation, scalable manufacturing, and clear regulatory pathways is essential to translate this promising technology into widespread clinical application. The integration of EV research into precision veterinary medicine and the One Health framework will further amplify their impact, contributing to improved animal welfare, food security, and public health.

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