Extracellular vesicles (EVs) are small, membrane-bound structures secreted by cells into the extracellular environment. These vesicles play an important role in intercellular communication, allowing cells to exchange information, molecules, and components without direct cell-to-cell contact. Different EVs have been identified, including exosomes, microvesicles (ie, ectosomes or shedding vesicles), and apoptotic bodies. These vesicles differ in biogenesis, size, content, release mechanisms, and biological functions. Moreover, EVs can contain diverse molecules, including proteins, lipids, nucleic acids, and metabolites. Recent evidence supports that EVs contain RNA, known as “exosomal shuttle RNA,” which can regulate the expression of genes in recipient cells at the mRNA level. Thus, exosomal

**OBJECTIVE**
Extracellular vesicles (EVs) derived from mesenchymal stromal cells (MSCs) are promising avenues in regenerative medicine, offering unique immunomodulatory and regenerative properties with lower immunogenicity. This study delves into the distinctive features of EVs extracted from feline adipose-derived MSCs (ASCs) and placenta-derived MSCs (PMSCs). The tissues were collected from 11 female cats aged between 4 and 7 years old.

**SAMPLE**
EVs extracted from MSCs from discarded fetal membranes from 7 female cats and SC adipose tissue from 11 cats.

**METHODS**
We comprehensively explored morphological characteristics, mitochondrial density, surface markers, and pro- and anti-inflammatory mediators, uncovering notable differences between ASCs and PMSCs.

**RESULTS**
Morphologically, ASCs exhibit a spindle-shaped form in contrast to the spherical morphology of PMSCs. Proliferation and clonogenic potential assessments reveal the faster proliferation and robust clonogenic nature of ASCs, suggesting their potential vital role in regenerative processes. Surface marker expression analysis indicates a significantly higher expression of multipotency-associated markers in ASCs, suggesting their superior proregenerative potential. Phenotyping of EVs demonstrates distinctive features, with CD9 expression suggesting varied EV secretion patterns. Notably, PMSCs exhibit superior CD81 expression, indicating their potential as preferred donors of mitochondria. Pro- and anti-inflammatory mediators analyzed at mRNA and microRNA levels reveal higher RNA content in EVs compared to source cells, emphasizing the potential of EVs in directing regenerative processes. Differential microRNA expression in EVs derived from ASCs hints at their regulatory roles in anti-inflammatory and immunometabolic processes.

**CLINICAL RELEVANCE**
This study lays a foundation for understanding the nuances between ASCs and PMSCs, which is crucial for harnessing the full therapeutic potential of MSCs and their EVs in tissue repair and regeneration.

**Keywords:** feline, exosome, stromal cell, regenerative medicine, extracellular vesicle

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shuttle RNA, as functional molecules, may modulate the functionality of recipient cells via delivery of a specific set of microRNA (miRNA), a subset of these RNAs found within exosomes. 3

Furthermore, recent findings in molecular biology stressed that EVs may also become a cargo for functional cell organelles like mitochondria, endoplasmic reticulum, and ribosomes. In addition to variations due to cell type, the specific cargo within EVs may vary depending on the physiological or pathological context. 6 These properties shed a promising light on their potential prorregenerative and immunomodulatory capabilities.

With that in mind, the identification and characterization of EVs require applying multiple complementary techniques to obtain a comprehensive understanding of their properties. Different techniques provide different types of information, and a combination of methods can help confirm the presence, size, content, and surface markers of EVs in a sample. The International Society of Extracellular Vesicles stated that minimal EV identification and characterization criteria include transmission electron microscopy or scanning electron microscopy size assessment, immunoblotting, flow cytometry, and sequencing. Although the sequences mentioned above are relatively simple for rodents or human cell-derived EVs, small animals, including cat-derived EVs, currently have limited options for such characterization. These limitations are primarily due to the lack of specific feline antibodies, a considerable technological obstacle that the development of functional tests may overcome.

In recent years, veterinary regenerative medicine has rapidly expanded as a unique field. The mesenchymal stromal cells (MSCs) derived from adipose tissue (ASCs) or placenta (PMSCs) belong to the most frequently used prorregenerative and immunomodulatory pool in the clinical practice of the stromal cells. Work completed over the past decade has demonstrated the beneficial effects of ASCs in feline regenerative medicine. 9–13 This may be explained by their multilineage differentiation potential, high expansion potential, and immunomodulatory effects. 14 MSCs, in general, have been shown to play a significant role in the modulation of immune system activity on different levels. Moreover, MSCs are a rich source of a wide range of growth factors, including transforming growth factor-β (TGF-β), IL-10, and prostaglandin E 2, which contribute to their immunomodulatory effects. 14 Finally, through IL-10, and prostaglandin E 2, which contribute to tissue damage and autoimmune diseases. Consequently, stromal cells’ anti-inflammatory, immune-stabilization, and antioxidative capacities fully justify their clinical application; however, whether their vesicles work similarly is still elusive. 16–17

EVs may be produced by these same tissues from which MSCs have been collected. Utilizing adipose and placental tissues as a source of EVs is attractive due to their promising therapeutic potential. 18–20 In addition, EVs have gained significant attention in recent years due to their potential critical roles in various physiological and pathological processes. 21–22 While there is limited knowledge about EVs derived from feline ASCs and PMSCs, the molecular signature of these EVs is particularly unclear, especially concerning their characteristics under normal and inflammatory conditions. In this study, we characterized EVs derived from feline ASCs and PMSCs under normal conditions and when stimulated with a cytokine cocktail.

For the first time, we demonstrate the distinct physiological characteristics of EVs derived from ASC and PMSC sources. This revelation prompts contemplation on the prospective clinical applications of these EVs in feline regenerative medicine.

**Methods**

**Isolation of placental-derived mesenchymal stromal cells and adipose-derived mesenchymal stromal cells**

After parturition, the discarded feline fetal membranes for PMSC isolation were collected from 7 female cats. All study procedures were reviewed and approved by the Environmental and Life Sciences University, Wroclaw, Poland. All owners consented to sample acquisition before collection. In addition, discarded SC adipose tissue (SAT) for ASC isolation was obtained from 11 adult cats undergoing routine spaying. All owners signed an informed consent. Amniotic membranes and SAT were washed with sterile PBS containing 1% antmyotic antibiotic solution (penicillin/streptomycin/amphotericin B) at room temperature, minced into small fragments using sterile scalpels blades, and subjected to enzymatic digestion with collagenase type I (1 mg/mL; Invitrogen) for 40 minutes at 37°C and 5% CO 2. Obtained homogenates were then filtered through 70-µm cell strainers, and the cell suspension was subjected to centrifugation at 1,200 X g for 10 minutes. Cell pellets of placental-derived mesenchymal stromal cells (PD-MSCs) and adipose-derived mesenchymal stromal cells (AD-ASCs) were washed with PBS and resuspended in Dulbecco modified Eagle medium (DMEM)/Ham F12 medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin solution and seeded in culture flasks. Cultures were maintained in a humidified CO 2 incubator at 37°C, and cells from passage 2 were used for the experiment.

**Analysis of surface markers using flow cytometry**

Surface markers of isolated PMSCs and ASCs were screened using flow cytometry. Cells were recovered using a 0.05% trypsin/EDTA solution, washed with PBS, and resuspended in a blocking buffer containing
Hank balanced salt solution (Sigma-Aldrich) supplemented with 5% fetal calf serum (Sigma-Aldrich). The live/dead cell numbers were measured using the cell counter AxioCell. Approximately 1 x 10⁶ cells were labeled with fluorescein/phycoerythrin/allophycocyanin-conjugated mouse monoclonal antibodies (Supplementary Tables S1-S3) specific for the feline mesenchymal markers CD44, CD90, CD105, CD14, and CD19 and hematopoietic markers, including CD45 and CD34, for 1 hour in the dark. Cells were then washed and fixed in a fluorescence-activated cell sorting fixation solution. The samples were analyzed using a Fortessa with FACSDiva version 9.0 flow cytometer equipped with an FCS Express 7.0 software (Becton Dickinson).

**Evaluation of cell morphology and proliferation capacity**

Cell morphology was assessed by confocal laser scanning microscopy (Observer 21 Confocal Spinning Disc V.2 Zeiss with live imaging chamber). One hundred cells were seeded onto glass coverslips and cultured for 24 hours under standard conditions in a complete culture medium (DMEM low glucose, 10% fetal bovine serum, and 1% penicillin and streptomycin). Mitochondria were stained with MitoRed fluorescent dye (1:1,000; Sigma-Aldrich) for 30 minutes at 37 °C. Cells were then fixed for 40 minutes at room temperature in 4% paraformaldehyde (PFA) solution, subjected to cell membrane permeabilization with a 0.1% Triton X-100 solution for 15 minutes, and incubated 40 minutes in the presence of atto-590-labeled phalloidin (1:800; Sigma-Aldrich) for 40 minutes at room temperature in the dark for cytoskeleton staining. The nuclei were counterstained using the diamidino-2-phenylindole (DAPI) in the ProLong Diamond Antifade Mountant with DAPI (Invitrogen). Photomicrographs were acquired with a Canon PowerShot camera and merged using ImageJ software (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation).

Cell proliferation capacity was assessed using the 5-bromo-2-deoxyuridine (BrdU) Cell Proliferation ELISA Kit (Abcam) according to the manufacturer’s recommendations, and horseradish peroxidase substrate degradation amount was measured with a fluorescence-activated cell sorting fixation solution. The samples were analyzed using a Fortessa with FACSDiva version 9.0 flow cytometer equipped with an FCS Express 7.0 software (Becton Dickinson). Cell proliferation capacity was assessed using the BrdU Cell Proliferation ELISA Kit (Abcam) according to the manufacturer’s recommendations, and horseradish peroxidase substrate degradation amount was measured with a fluorescence-activated cell sorting fixation solution. The samples were analyzed using a Fortessa with FACSDiva version 9.0 flow cytometer equipped with an FCS Express 7.0 software (Becton Dickinson). Cell proliferation capacity was assessed using the BrdU Cell Proliferation ELISA Kit (Abcam) according to the manufacturer’s recommendations, and horseradish peroxidase substrate degradation amount was measured with a fluorescence-activated cell sorting fixation solution. The samples were analyzed using a Fortessa with FACSDiva version 9.0 flow cytometer equipped with an FCS Express 7.0 software (Becton Dickinson).

**Analysis of mRNA and miRNA**

The gene expression of inflammation-related markers (indoleamine-2,3 dioxygenase 1 [IDO1], TGF-β1, IL-10, and IL-4) and miRNAs (miR-17-5p, miR-10a-3p, miR-21-5p, and miR-146a-5p) was analyzed using the quantitative reverse transcriptase PCR (RT-qPCR) technique. Exosomal RNAs were isolated using the miRNeasy Micro Kit (Qiagen) and quantified using a NanoDrop spectrophotometer. cDNA was synthesized from a total amount of 500 ng RNA using either the Tetro cDNA Synthesis Kit (Bioline Reagents Ltd) for mRNA and miRCURY LNA RT Kit (Meridian Bioscience) for miRNA in a T100 thermal cycler (Bio-Rad). qPCR reactions were prepared with the SensiFAST SYBR & Fluorescein Kit (Meridian Bioscience) and ran in a CFX Connex Real-Time PCR Detection System (Bio-Rad). The levels of transcripts were normalized using the GAPDH for mRNA and snU6 for miRNA.

**Statistical analysis**

Data were analyzed using the GraphPad Prism software (Prism 9). Statistical comparison between the experimental groups was calculated with the one-way ANOVA followed by unpair Student or Tukey test for post hoc comparison. Differences were considered statistically significant at P < .05, P < .01, P < .001, and P < .0001. Results are presented as mean ± SD.

**Results**

**Feline PMSC and ASC cytobiological phenotype**

The isolation of MSCs from cat amniotic membrane and SAT resulted in homogeneous populations of PMSCs and ASCs exhibiting typical fibroblastic, spindle-shaped morphology for ASCs and a somewhat more spherical appearance for PMSCs with...
adherence capacity to plastic culture dish under standard culture conditions. ASCs were characterized by prominent ovoid nuclei and parallel fibrillary assembly of F-actin filaments extending across the elongated cell body. By contrast, PMSCs displayed smaller flattened cellular bodies, only a few long and slender F-actin processes with unorganized microtubule structures within a diffuse cytoskeleton. Nuclei appeared either ovoid or rounded (Figure 1). MitoRed rhodamine-based staining evidenced a well-developed mitochondrial network uniformly distributed around the perinuclear site in both cell populations. Noteworthy, ASCs were characterized by the dominance of fused elongated mitochondria. At the same time, PMSCs exhibited a high occurrence of globular immature mitochondria as an indicator of active mitochondrial dynamism. Fluorescence-activated cell sorting immunophenotyping showed that both PMSCs and ASCs were positive for the MSC surface markers CD90 and CD105, adhesion protein CD44. They were negative for CD34 and CD45, which excluded their hematopoietic origin. Interestingly, ASCs exhibited a higher abundance of CD90, CD105 ($P < .0001$), and CD44 ($P < .01$) compared to PMSCs expressed by significantly higher color reactions.

Similarly, ASCs were found to possess greater fibroblast-like properties (Figure 1), as evidenced by the significantly superior number of colonies compared to PMSCs, with lower clonogenic potential ($P < .05$). Similarly, ASCs manifested a visible more robust

![Figure 1](image_url)

**Figure 1**—Cytobiological characterization of isolated feline placental-derived mesenchymal stromal cells (PD-MSCs) and adipose derived mesenchymal stromal cells (AD-MSCs). A—Representative photomicrographs of cells observed under a bright-field inverted microscope and a confocal microscope after staining with the diamidino-2-phenylindole (DAPI), phalloidin, and MitoRed fluorescent dyes. Scale bar = 250 and 20 µm. B—Bar charts depicting the percentage of MSCs positive for CD105, CD90, CD44, and CD19 determined using fluorescence-activated cell sorting analysis. C—A signal representative of stained marker phycoerythrin (CD45) and CD35) was obtained with flow cytometry. D—Representative clonogenic assay wells with cells stained with pararosaniline and histograms showing the percentage of the ability to form colonies (CFU-Fs) for each cell population. E—Histograms demonstrating the absorbance levels of incorporated 5-bromo-2-deoxyuridine (BrdU) through horseradish peroxidase substrate degradation. Representative data are shown as mean ± SD. *$P < .05$, **$P < .01$, ****$P < .0001$, comparison between AD-MSCs and PD-MSCs. ns = Not significant.
proliferative capacity with increased BrdU incorporation into neosynthesized DNA, suggesting a higher number of cells undergoing the S-phase of the cell cycle. PMSCs were characterized by a lower proliferative rate illustrated with dropped BrdU assimilation, as an indicator of reduced DNA biosynthesis and cell division when compared to adipose-derived mesenchymal stromal cells (AD-MSCs) \( (P < .01) \).

**Characterization of EVs derived from PMSCs and ASCs**

The average protein concentration of purified EVs normalized to 1 mL of CCM is represented (Figure 2). CCM derived from PMSCs produced the highest amount of EV proteins compared to ASCs, with no statistically significant difference. A homogeneous distribution of fluorescently labeled EVs has been observed, suggesting a comparable particle size and number between both studied populations. Flow cytometry analysis of surface tetraspanin molecules further revealed a higher positive signal for CD63 and a low signal for CD9 and CD81 in both EV populations. Interestingly, EVs derived from ASC CCM exhibited a higher abundance of CD9 contrasted by a reduced expression of CD81 compared to EVs isolated from PMSC CCM \( (P < .01; P < .05) \). Moreover, the levels of CD63 markers were similar in both EV samples. Transmission electron microscopy confirmed the purity of analyzed specimens and the presence of abundant individual spherical nanoparticles homogeneously distributed within the analysis fields for either ASC or PMSC EVs.

**Anti-inflammatory payload expression analysis**

The gene expression analysis of critical immunomodulators has been established using RT-qPCR in ASCs, PMSCs, and their respective EVs. Under standard conditions, both resting unstimulated MSCs isolated from the placenta and SAT exhibited similar

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**Figure 2**—Characterization of isolated adipose-derived mesenchymal stromal cells (AD-MSCs) and placental-derived mesenchymal stromal cells (PD-MSCs) and derived extracellular vesicles (EVs). A—Absolute protein concentration of purified EV samples. B—Calculated average number of nanoparticles detected by the fluorescence-activated cell sorting technique. C—Representative plots of gated EVs stained for fluorescein (CD63) and phycoerythrin (CD81 and CD9) using flow cytometry. D—Bar chart depicting the percentage of EVs positive for surface tetraspanin molecules. E—Representative transmission electron micrographs showing the typical morphology and purity of isolated EVs. Representative data are shown as mean ± SD. \( ^*P < .05, ^{**}P < .01 \), comparison between AD-MSCs EVs and PD-MSCs EVs.
expression levels of IDO1, TGF-β1, IL-10, and IL-4 anti-inflammatory cytokines as well as immunoregulatory miRNAs, including miR-17-5p, miR-10a-3p, miR-21-5p, and miR-146a-5p (Figure 3). Importantly, the levels of the analyzed anti-inflammatory cytokines transcripts and miRNAs were found significantly higher in EVs derived from ASCs and PMSCs in comparison to total producing cells \( (P < .0001) \), except for miR-17-5p and miR-21-5p whose levels were detected at lower significance in PMSC EVs than in cell-derived total miRNAs \( (P < .0001) \). Noteworthy, the EVs purified from ASC CCM were characterized by considerable richness in selected IDO1, TGF-β1, IL-10, IL-4, miR-17-5p, miR-10a-3p, miR-21-5p, and miR-146a-5p immunomodulatory mediators conversely to EVs derived from PMSCs in which visible lower levels of the same transcripts were noted \( (P < .0001) \), suggesting that ASCs might represent a superior source of anti-inflammatory EVs as compared to PMSC EVs.

**Discussion**

EVs derived from MSCs hold promising potential in regenerative medicine due to several unique characteristics, such as their ability to immunomodulate and promote regeneration while having lower immunogenicity.\(^{24-27}\) In this study, we explore the contrasting features of EVs from ASCs and PMSCs, shedding light on their morphology, mitochondrial density, surface marker expressions, as well as their pro- and anti-inflammatory mediators.

**Morphological distinctions**

The observed spindle-shaped morphology in ASCs over 2 passages starkly contrasts with the more spherical morphology of PMSCs. Although the morphology of MSCs can be influenced by various factors, including the culture substrate, growth factors, and differentiation cues, both cells were subject to the same conditions. MSCs cultured during early passages might display a more rounded morphology before assuming their characteristic fibroblast-like shape, which has been reported for MSCs derived from the canine placenta.\(^ {28}\) Alternatively, this could represent a subpopulation of MSCs with more gradual proliferation than spindle-shaped cells.\(^ {29}\) This is consistent with the current knowledge of ASC, which is known to proliferate faster and possess more considerable clonogenic potential.\(^ {30,31}\) Regardless, this morphology serves as a visual identifier and correlates with the expression of surface markers. Moreover, mitochondrial staining reveals a higher density in PMSCs, suggesting superior survival potential and making them a preferable source for mitochondrial isolation.\(^ {32}\)

**Proliferation and clonogenic potential**

ASCs demonstrate faster proliferation and possess considerable clonogenic potential, a vital
factor in assessing MSC functionality compared to PMSCs. This finding is consistent with historical data obtained in cats for ASCs when comparing these to bone marrow–derived MSCs. Additionally, these findings are consistent with the population doubling time described for canine PMSCs, for which a rise between the second and third passages has been noted, but this remains constant until, in this case, passages 5 to 10. The ability to form colonies has long been recognized as a fundamental parameter, and our findings underscore the robust clonogenic nature of ASCs, supporting their pivotal role in regenerative processes. Amniotic fluid and umbilical cord MSCs showed a significant increase in population doubling time in dogs, and further investigation is necessary to determine if these may prove to be more reliable sources in cats.

Surface marker expression

Surface markers associated with multipotency, including CD44, CD90, and CD105, showed a significantly higher expression in ASCs than in PMSCs. Expression of CD19 is negligible, which is also consistent with the minimal criteria for defining multipotent MSCs as noted from the position statement by the International Society for Cellular Therapy. This elevated expression hints at the more significant preregenerative potential of ASCs, emphasizing their versatility in therapeutic applications. The surface marker profile suggests that ASCs may be more adept at promoting regenerative responses than PMSCs.

EV phenotyping

The phenotyping of EVs derived from ASCs and PMSCs offers intriguing insights into their potential functions. CD63 and CD9 are members of the tetraspanin family and are highly enriched within EV membranes. These surface receptors are suspected to regulate the EV uptake and delivery process. However, CD9 and CD63 have been individually associated with specific functions. CD9 exhibits lower expression in PMSCs, suggesting a reduced secretion of EVs from MSCs originating from placental tissues compared to adipose tissues. CD9 also regulates the expression and activation of adhesion molecules in different cells of the immune system as well as endothelial cells, ultimately resulting in the activation of T lymphocytes. This quality may also be enhanced in ASCs as compared to PMSCs. An equal expression of CD63 between these EVs from ASCs and PMSCs was also found. CD63 is also known for activating platelets under inflammation, which may indicate comparable activator functions in both cell types. In recent findings, microvesicles originating from MSCs, a subset of EVs released through membrane budding, have been identified to harbor mitochondrial components, encompassing proteins and mitochondrial DNA. Specifically, the mitochondrial protein MTCO2, or cytochrome c oxidase subunit II, derived from the mitochondrial genome, was observed in density gradient fractions coinciding with the localization of the EV marker CD81.

Notably, the superior expression of CD81 in PMSCs may suggest their potential as preferred donors for mitochondrial studies. Mitochondrial components, such as mitochondrial DNA and N-formyl peptides, signal cellular damage. They activate inflammatory responses through pathways involving the NLR family pyrin domain containing 3 inflammasome, Toll-like receptor-9, and proinflammatory gene expression, while mitochondrial metabolites and proteins further contribute to inflammation and immune responses. Consequently, through their mitochondrial contents, PMSCs may aid in regulating innate and adaptive immunity.

Pro- and inflammatory mediators at mRNA and miRNA levels

A comprehensive analysis of pro and inflammatory mediators, including IDO1, TGF-B1, IL-4, IL-10, miR-17-5p, miR-21-5p, miR-146a-5p, and miR-10a-3p, at the mRNA and miRNA levels, for EVs from both ASCs and PMSCs was also performed. Notably, EVs consistently demonstrate higher RNA content compared to their source cells. This finding underscores the potential value of EVs in directing regenerative processes, acting as carriers of a diverse array of molecular cargo. This is consistent with findings of transcriptomic studies from immune cell–derived vesicles in which a substantial portion of the RNAs exhibited enrichment in the EVs compared to the cellular RNA. This further suggests a selective process by which cells earmark specific RNAs for extracellular release, and many of these have been identified as evolutionarily conserved sequences and linked to gene regulatory functions. Facilitated by EV-mediated transfer, the horizontal exchange of RNA among cells enables the distribution of genetically encoded messages capable of influencing the function of target cells, and this may be one of the pathways by which EVs exert an effect.

miRNA expression

Within EVs, a higher expression of miRNAs is observed in ASCs. miRNAs are recognized for their regulatory roles, and this differential expression hints at the potential of ASC-derived EVs in modulating anti-inflammatory and immunometabolic processes. On the other hand, PMSCs have demonstrated a notable impact on the antigen-presenting capacity of mononuclear cells and dendritic cells in a manner distinct from cord-derived MSCs, leading to a discernible decrease in T-cell proliferation. Concomitantly, elevated levels of IL-10 and TGF-B1 were observed, coupled with a reduction in IFNγ levels and an enrichment of CD3+ CD4+ CD25+ T-regulatory cells. The molecular functions of these miRNAs deserve further exploration, as they could be critical players in directing therapeutic responses from both cell types.

Although ASCs may be more advantageous demonstrating faster proliferation, higher clonogenic potential, higher expression of surface markers, and higher expression of miRNAs as well as having the potential for autologous harvest and use
over PMSCs, infection with foamy virus leading to proliferation arrest has been reported making them a less ideal solution. Ultimately, the goal is to source the desirable properties of these cells in a safe, efficacious, sustainable, and scalable way.43 EVs may circumvent some of the challenges encountered with MSCs, including potential immunogenicity, off-target homing, tumorigenicity, heterogeneity, and storage and handling, thereby affecting the scalability and sustainability of this treatment. This initial study serves as a foundation for future investigations. The observed distinctions open avenues for further research, particularly in understanding the specific roles of EVs in modulating anti-inflammatory and immunometabolic processes. The comparison between ASCs and PMSCs provides valuable insights into their regenerative potential, guiding the development of tailored therapeutic approaches.

In conclusion, our study unveils the distinctive attributes of ASCs vs. PMSCs, highlighting their morphological disparities, mitochondrial characteristics, proliferation capacities, and surface marker expressions. These findings underscore the diverse potential applications of ASCs and PMSCs in regenerative medicine. Moreover, the phenotype of EVs derived from these MSCs sheds light on their potential functions, paving the way for future studies on their specific roles in therapeutic interventions. As we navigate the evolving landscape of regenerative medicine, understanding these nuances becomes crucial for harnessing the full potential of MSCs and their EVs in promoting tissue repair and regeneration.

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Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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### Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org