

# The Growing Potential of Extracellular Vesicle Research in Veterinary Medicine

# Naraščajoči potencial raziskav zunajceličnih veziklov v veterinarski medicini

## Key words

extracellular vesicles;  
regenerative veterinary  
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Extracellular vesicles (EVs) are small, information-rich subcellular structures that have attracted considerable attention in the scientific community. Their ubiquitous presence in the body and their natural role as cellular messengers make them valuable indicators of the physiological and pathological state of cells and tissues. Currently, the number of specific studies on the presence, properties and role of EVs in all life forms is increasing exponentially. In veterinary medicine, EVs are still relatively under-researched, and we have only recently begun to explore their potential.

EVs facilitate communication between cells or tissues. They are information carriers that travel from cell of origin to the target cells via body fluids. Their diverse cargo includes a plethora of proteins, lipids, DNA and other small nucleic acids, all of which reflect the state of the donor cell (1). As a result, EV research is rapidly expanding, particularly within the One Health concept, which aims at interlinked health outcomes for humans, animals and the environment (2). The integrated approach of the One Health concept emphasizes the importance of the link between veterinary and human medicine. Research on selected animal models provides valuable insights that can be transferred to human research in many clinical areas, and vice versa (3). Key areas of interest include understanding the role of EVs in physiology

Zunajcelični vezikli (ZV) so majhne, informacijsko bogate subcelične strukture, ki so v znanstveni skupnosti pritegnile veliko pozornosti. Zaradi njihove vsesplošne prisotnosti v telesu in njihove naravne vloge kot prenašalci informacij so dragoceni pokazatelji fiziološkega in patološkega stanja celic in tkiv. V tem trenutku število študij o prisotnosti, lastnostih in vlogi ZV v vseh oblikah življenja eksponentno narašča. V veterinarski medicini so ZV še vedno razmeroma slabo proučeni in šele pred kratkim smo začeli bolj natančno raziskovati njihov potencial.

ZV omogočajo komunikacijo med celicami ali tkivi. So nosilci informacij, ki prek telesnih tekočin potujejo od matične do tarčnih celic. Njihov raznovrsten tovor vključuje številne proteine, lipide, DNA in druge majhne nukleinske kisline, ki odražajo stanje celice, iz katere izvirajo (1). Zato se raziskave ZV hitro širijo, zlasti v okviru koncepta Eno zdravje, ki obravnava medsebojno povezanost zdravja ljudi, živali in okolja (2). Celostni pristop koncepta Eno zdravje poudarja pomen povezave med veterinarsko in humano medicino. Raziskave na izbranih živalskih modelih zagotavljajo dragocena spoznanja, ki jih je mogoče prenesti na številna področja raziskav v humani medicini, in obratno (3). Ključna področja zanimanja vključujejo razumevanje vloge ZV v fiziologiji in patofiziologiji, potencialno uporabo

and pathophysiology, potential use as disease biomarkers, exploring their potential as therapeutics or vaccine carriers, and applications in regenerative veterinary medicine (4). Currently, EV research is focused on cattle, pigs, dogs, birds, horses and cats, as well as some small ruminants and exotic animals (5).

Despite tremendous progress in a short period of time, some critical challenges remain if we are to take EV research in veterinary medicine to the next level. These include the standardization of isolation and characterization methods, the optimization of dosing and storage conditions, and the identification of species-specific EV markers and reference genes. In contrast to EV research in human medicine, where many protocols are already established, there is a greater variability in starting materials for EV isolation in veterinary applications, which makes the transfer of protocols difficult. In addition, there are few functional studies on EVs in animals (5). The challenges are being relentlessly addressed by the scientific community, particularly thorough the work of the International Society for Extracellular Vesicles (ISEV), which is continuously updating minimal requirements for standardization of workflows in this field (6).

The lipid bilayer envelope of EVs ensures the stability of their cargo, making them excellent candidates for non-invasive diagnostics. EVs can be isolated from various minimally invasive samples, such as blood, urine or saliva. Their cargo reflects the state of the donor cell and enables early detection and monitoring of diseases in different areas of veterinary medicine (7). Tumor-derived EVs contain specific proteins and RNAs that can be used to differentiate between healthy and tumor cells or even between different tumor phenotypes. Zamboni and colleagues (8) showed a correlation between elevated levels of miR-21-5p in EVs from canine plasma and mast cell tumors. Using bioinformatic analysis of RNA sequencing data, Özmen and colleagues (9) also identified miR-21-5p as a potential marker for hepatic hemangiosarcoma in dogs. EVs may contain pro-inflammatory cytokines and microRNAs indicative of inflammatory conditions. In horses, the presence of pro-inflammatory cytokines such as TNF- $\alpha$  or IL-6 in synovial fluid EVs is associated with joint inflammation and arthritis and could be used to monitor disease status (10, 11). EVs may contain specific pathogen-derived molecules that allow identification of infections. For example, Palacios and colleagues (12) showed that mycobacteria-derived EV-associated lipoprotein LpqH in blood samples can help distinguish between *Mycobacterium bovis* and *Mycobacterium avium* subsp. *paratuberculosis* infection. We can use EVs to detect organ damage or stress in specific tissues. NGAL has been presented as a promising biomarker of kidney damage in canine urine (13), and in humans, Ugarte and colleagues (14) have already linked the presence of EV-derived NGAL to diabetic kidney disease. Similarly, liver damage in pets can be detected clinically by determining various hepatic markers in blood, but further studies are needed to link them to EVs and evaluate which markers are most suitable for the early detection of liver failure. Elevated

kot biomarkerjev bolezni, raziskovanje njihovega potenciala za prenos in tarčno dostavo zdravil ali kot nosilcev cepiv ter uporabo v regenerativni veterinarski medicini (4). Trenutno so raziskave ZV osredotočene na govedo, prašiče, pse, ptice, konje in mačke ter nekatere male prežvekovalce in eksotične živali (5).

Kljub izjemnemu napredku področja v kratkem času, ostajajo nekateri kritični izzivi nerešeni, predvsem, če želimo raziskave ZV v veterinarski medicini dvigniti na naslednjo raven. Ti vključujejo standardizacijo metod izolacije in karakterizacije, optimizacijo pogojev doziranja in shranjevanja ter opredelitev vrstno specifičnih označevalcev ZV in referenčnih genov. V nasprotju z raziskavami ZV v humani medicini, kjer so številni protokoli že uveljavljeni, so izhodni materiali za izolacijo EV v veterini bolj raznoliki, kar otežuje direktni prenos protokolov. Poleg tega je število funkcionalnih študij o ZV pri živalih nizko (5). Znanstvena skupnost se s temi izzivi vztrajno ukvarja, pomembne temelje pa postavlja Mednarodno združenje za zunajcelične vezikle (ISEV), ki nenehno posodablja minimalne zahteve za standardizacijo delovnih postopkov na tem področju (6).

Lipidni dvosloj ZV zagotavlja stabilnost njihovega tovora, zato so odlični kandidati za neinvazivno diagnostiko. ZV lahko izoliramo iz različnih minimalno invazivnih vzorcev, kot so kri, urin ali slina. Njihov tovor odraža stanje starševske celice in omogoča zgodnje odkrivanje in spremljanje bolezni na različnih področjih veterinarske medicine (7). ZV, ki izvirajo iz tumorjev, vsebujejo specifične proteine in RNA molekule, ki jih je mogoče uporabiti za razlikovanje med zdravimi in tumorskimi celicami ali celo med različnimi tumorskimi fenotipi. Zamboni in sodelavci (8) so pokazali povezavo med povišanimi ravnmi miR-21-5p v ZV iz plazme psov in tumorjev mastocitov. Özmen in sodelavci (9) so z bioinformacijsko analizo podatkov sekvenciranja RNA prav tako identificirali miR-21-5p kot potencialni označevalec prisotnosti jetrnega hemangiosarkoma pri psih. ZV lahko vsebujejo vnetne citokine in mikroRNA, ki kažejo na prisotnost vnetnega stanja. Pri konjih je prisotnost vnetnih citokinov, kot sta TNF- $\alpha$  ali IL-6, v ZV sinovialne tekočine povezana z vnetjem sklepov in artritom ter se lahko uporablja za spremljanje stanja bolezni (10, 11). ZV lahko vsebujejo specifične molekule, ki izvirajo iz patogenov in omogočajo identifikacijo okužb. Npr. Palacios in sodelavci (12) so pokazali, da lahko z mikobakterijami povezan lipoprotein LpqH iz ZV v vzorcih krvi pomaga razlikovati med okužbo z *Mycobacterium bovis* in *Mycobacterium avium* subsp. *paratuberculosis*. ZV lahko uporabimo za odkrivanje poškodb organov ali stresa v določenih tkivih. NGAL je bil predstavljen kot obetaven biomarker poškodbe ledvic v pasjem urinu (13), pri ljudeh pa so Ugarte in sodelavci (14) prisotnost NGAL iz ZV že povezali z diabetično ledvično boleznijo. Podobno lahko poškodbe jeter pri hišnih ljubljenceh klinično zaznamo z določanjem različnih jetrnih označevalcev v krvi, vendar so potrebne nadaljnje študije za njihovo povezavo z ZV in oceno, kateri označevalci so najprimernejši za zgodnje odkrivanje odpovedi jeter. Pri različnih možganskih motnjah so opazili

levels of the protein S100B in CSF have been observed in various brain disorders and are therefore gaining attention as a possible biomarker for neuroinflammation in animals (15). Piibor and colleagues (16) investigated the association between EVs in the uterine fluid and reproductive disorders in cows and specifically showed differences in EV protein profiles between healthy cows and cows with subclinical or clinical endometritis. These are only a few examples as several other potential biomarkers from different body fluids are being investigated in basic research and clinical studies in different animal species.

Another interesting research direction in the field is the use of EVs in different therapeutic approaches. EVs can be loaded or modified to deliver therapeutic agents – drugs, proteins or nucleic acids – directly to the target tissue (17). While the natural origin of EVs reduces the risk of immune reactions, the transferred bioactive molecules can influence proliferation, differentiation or apoptosis in target tissues (18). By introducing carefully selected modifications, we can bind specific ligands to the EV surface, enhance their natural abilities, optimize delivery to target cells and reduce off-target effects (19). Many applications include the treatment of cancer, infections and various inflammatory diseases. EVs from immune cells can modulate the immune response, offering new potential treatments for autoimmune diseases, allergies and other immune-related diseases in animals. Many current treatments are based on symptom relief, but EVs offer an alternative treatment focused on influencing the cause of the disease. Thanks to their natural presence in the body, EVs are less likely to trigger an immune response, especially compared to some synthetic nanoparticles. Since EVs can be derived from autologous cells, there is less risk of rejection. This also opens numerous possibilities for personalized treatments (20). Of particular interest is the investigation of the influence of EVs on fertility enhancement, e.g. in economically important animals. As EVs contribute to the regulation of sperm and oocyte maturation and are an important part of the embryonic development microenvironment, we can use them to support embryonic development and improve reproductive outcomes in assisted reproductive techniques (21). It has also been shown that different cryopreservation protocols for sperm and oocytes need to be thoroughly investigated and improved (22, 23). For example, Pedrosa and colleagues (24) recently showed that certain miRNAs present in EVs from seminal plasma indicate low sperm cryotolerance in boars and may serve as predictive factors for the success of the cryopreservation process.

The development of vaccines based on EVs is promising from several points of view. EVs are natural carriers of biomolecules, and the lipid bilayer suitably protects the cargo. Novel techniques for vaccine production aim at products with a better safety profile and targeted immunogenicity. EVs are superior to synthetic nanoparticles in this respect, and their small size even allows them to overcome certain biological barriers that were previously considered insurmountable (25). Furthermore, novel vaccine production options represent an

povišane ravni proteina S100B v cerebrospinalni tekočini, zato so le-ta omenja kot možen biomarker nevrovnetja pri živalih (15). Piibor in sodelavci (16) so raziskovali povezavo med ZV v maternici in reproduktivnimi motnjami pri kravah ter posebej pokazali razlike v proteinskih profilih ZV med zdravimi kravami ter kravami s subkliničnim ali kliničnim endometritisom. Našteti je le nekaj primerov. Še veliko več drugih potencialnih biomarkerjev, prisotnih v ZV iz različnih telesnih tekočin, se preučuje v temeljnih raziskavah in kliničnih študijah na različnih živalskih vrstah.

Druga zanimiva raziskovalna smer na tem področju je uporaba ZV v različnih terapevtskih pristopih. ZV lahko izboljšamo oz. spremenimo tako, da omogočajo tarčno dostavo terapevtskih snovi - zdravila, proteine ali nukleinske kisline - neposredno v ciljno tkivo (17). Medtem, ko naravni izvor ZV zmanjšuje tveganje za pojav neželenih imunskih reakcij, lahko prenesene bioaktivne molekule vplivajo na proliferacijo, diferenciacijo ali apoptozo v tarčnem tkivu (18). Z uvajanjem skrbno izbranih modifikacij, lahko na površino ZV vežemo specifične molekule, povečamo njihove naravne sposobnosti, optimiziramo dostavo v ciljne celice in zmanjšamo neželene učinke (19). Številne aplikacije vključujejo zdravljenje raka, okužb in različnih vnetnih bolezni. ZV imunskih celic lahko modulirajo imunski odziv, kar ponuja nove možnosti zdravljenja avtoimunskih bolezni, alergij in drugih, z imunskim sistemom povezanih bolezni pri živalih. Številna trenutna zdravljenja teh obolenj temeljijo predvsem na lajšanju simptomov, ZV pa ponujajo alternativo, ki se osredotoča na zdravljenje vzroka bolezni. Zaradi svoje naravne prisotnosti v telesu ZV manj verjetno sprožijo imunski odziv, zlasti v primerjavi z nekaterimi sintetičnimi nanodelci. Ker lahko ZV pridobivamo iz avtolognih celic, je tveganje zavrnitvene reakcije manjše. To odpira tudi številne možnosti za personalizirano zdravljenje (20). Posebno zanimive so raziskave vpliva ZV na izboljšanje plodnosti, npr. pri gospodarsko pomembnih živalih. Ker ZV prispevajo k uravnavanju zorenja spermijev in jajčnih celic ter so pomemben del mikrookolja embrionalnega razvoja, jih lahko uporabimo za podporo embrionalnega razvoja in izboljšanje reproduktivnih rezultatov pri tehnikah oploditve z biomedicinsko pomočjo (21). Obstaja potreba po raziskavah na področju optimizacije protokolov za kriokonzervacijo spermijev in jajčec (22, 23). Pedrosa in sodelavci (24) so na primer nedavno pokazali, da nekatere miRNA molekule, ki so prisotne v ZV v semenski plazmi, kažejo na nizko stopnjo kriotolerance sperme pri merjascih in lahko služijo kot napovedni dejavnik za uspešnost postopka krioprezervacije.

Razvoj cepiv, ki temeljijo na ZV, je obetaven z več vidikov. ZV so naravni nosilci biomolekul, lipidni dvosloj pa ustrezno ščiti tovor. Eden izmed pomembnih ciljev novih tehnik za proizvodnjo cepiv je priprava izdelkov z boljšim varnostnim profilom in usmerjeno imunogenostjo. V tem pogledu so ZV boljši od sintetičnih nanodelcev, njihova velikost pa jim omogoča premagovanje nekaterih bioloških ovir, ki so prej veljale za nepremostljive (25). Nadalje, nova cepiva predstavljajo alternativo uporabi antibiotikov (26). Zmanjšanje

alternative to the use of antibiotics (26). Reducing the use of antibiotics is an urgent issue in animal husbandry, food-producing species and overall, which is closely linked to the One Health initiative.

Last, but not least, in regenerative veterinary medicine efforts are shifting towards cell-free therapies, focusing on the need for readily available off-the-shelf products. Such products offer more safety and customized treatments. Compared to cell-based therapies, EVs offer several advantages (27). EVs derived from medicinal/mesenchymal stem cells are of particular interest. It is now clear that EVs are the cellular fraction of medicinal/mesenchymal stem cells that influence immunomodulation of damaged tissue.

The EV cargo is protected in body fluids, which is reflected in and extended shelf life of EV-based products. On the other hand, preparations from living cells require stringent viability conditions that are not easy or cheap to maintain.

Their anti-inflammatory properties may reduce the need for long-term medication. Also, boosting immune response can be helpful in cancer therapies or in fighting infections. An important factor compared to cell therapies is the lower risk of tumor formation, as EVs do not carry the risk of uncontrolled cell proliferation (5, 28). All these positive properties favor the active research of EVs, which are used in various areas of regenerative veterinary medicine, e.g. neurodegeneration, joint and cartilage diseases, wound healing and skin regeneration, autoimmune diseases and fertility therapies (29).

While EVs offer immense promises and applications, challenges such as standardizing isolation techniques, optimizing the high cost/low yield ratio, improving detection sensitivity and performing species-specific studies still need to be addressed to unlock their full potential in veterinary medicine. A new era has begun, and the increasing number of publications highlights the promising applications of EVs in non-invasive diagnostics, targeted delivery of molecules and regenerative treatments. With their remarkable versatility, EVs are uniquely positioned to revolutionize the way we think about veterinary medicine.

uporabe antibiotikov je pomembna iniciativa v živaloreji, pri živalskih vrstah za proizvodnjo hrane in je hkrati tesno povezano s pobudo Eno zdravje.

Nenazadnje se na področju regenerativne veterinarske medicine prizadevanja usmerjajo v uvedbo brezceličnih terapij, pri čemer se osredotočamo na potrebo po lahko dostopnih izdelkih, ki so na voljo za takojšnjo uporabo v kliniki. Želimo, da takšni izdelki zagotavljajo večjo varnost in omogočajo zdravljenje po meri. V primerjavi s celičnimi terapijami imajo ZV več prednosti (27). ZV, pridobljene iz medicinskih/mezenhimskih matičnih celic, so še posebej zanimivi. Zdaj je jasno, da so ZV tista celična frakcija medicinskih/mezenhimskih matičnih celic, ki vpliva na imunomodulacijo poškodovanega tkiva.

Tovor ZV je v telesnih tekočinah zaščiten, kar se odraža v podaljšanem roku uporabnosti izdelkov na osnovi ZV. Po drugi strani pa pripravki iz živih celic zahtevajo stroge pogoje vzdrževanja viabilnosti, ki jih je težko in ekonomsko neučinkovito vzdrževati.

Protivnetne lastnosti ZV lahko zmanjšajo potrebo po dolgotrajni uporabi zdravil. Spodbujanje delovanja imunskega odziva je lahko koristno pri zdravljenju raka ali v boju proti okužbam. Pomemben dejavnik v primerjavi s celičnimi terapijami je manjše tveganje za nastanek tumorjev, saj ZV ne prinašajo tveganja nenadzorovane proliferacije celic (5, 28). Vse te pozitivne lastnosti spodbujajo aktivne raziskave na področju ZV, ki izkazujejo potencial za uporabo na različnih področjih regenerativne veterinarske medicine, npr. pri nevrodegeneraciji, boleznih sklepov in hrustanca, celjenju ran in regeneraciji kože, avtoimunskih boleznih in zdravljenju plodnosti (29).

Čeprav so ZV izjemno obetavni in uporabni, je treba za sprostitve njihovega celokupnega potenciala v veterinarski medicini še vedno obravnavati izzive, kot so standardizacija tehnik izolacije, optimizacija razmerja med visokimi stroški in nizkimi donosi, izboljšanje občutljivosti zaznavanja in izvajanje študij, specifičnih za posamezne vrste. Začelo se je novo obdobje in vedno večje število publikacij poudarja obetavno uporabo ZV v neinvazivni diagnostiki, ciljani dostavi molekul in regenerativnem zdravljenju. Zaradi svoje izjemne vsestranskosti so ZV v edinstvenem položaju, da korenito spremenijo naš pogled na veterinarsko medicino.

## References

- van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol* 2018; 19(4): 213-28. doi: 10.1038/nrm.2017.125
- Espejo C, Ezenwa VO. Extracellular vesicles: an emerging tool for wild immunology. *Discov Immunol* 2024;3(1):kyae011. doi: 10.1093/discim/kyae011
- Tozon N. Translation Oncology through the One Health Perspective. *Slov Vet Res* 2025; 62(Suppl. 27): 9–12. doi: 10.26873/SVR-2180-2024
- Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015; 4: 27066. doi: 10.3402/jev.v4.27066
- Moccia V, Sammarco A, Cavicchioli L, Castagnaro M, Bongiovanni L, Zappulli V. Extracellular Vesicles in Veterinary Medicine. *Animals (Basel)* 2022; 12(19): 2716. doi: 10.3390/ani12192716
- Welsh JA, Goberdhan DCI, O'Driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles* 2024; 13(2): e12404. doi: 10.1002/jev2.12404
- Kumar MA, Baba SK, Sadida HQ, et al. Extracellular vesicles as tools and targets in therapy for diseases. *Signal Transduct Target Ther* 2024; 9(1): 27. doi: 10.1038/s41392-024-01735-1
- Zamboni C, Zamarian V, Stefanello D, et al. Plasma small extracellular vesicles from dogs affected by cutaneous mast cell tumors deliver high levels of miR-21-5p. *Front Vet Sci* 2022; 9: 1083174. doi: 10.3389/fvets.2022.1083174
- Özmen Ö, Kaya B, Karaman K. Transcriptome analysis and bioinformatics characterization of canine hemangiosarcoma: potential therapeutic targets. *Slov Vet Res* 2025; 62: 41–52. doi: 10.26873/SVR-1797-2024
- Elashry MI, Speer J, De Marco I, Klymiuk MC, Wenisch S, Arnhold S. Extracellular vesicles: a novel diagnostic tool and potential therapeutic approach for equine osteoarthritis. *Curr Issues Mol Biol* 2024; 46(11): 13078–104. doi: 10.3390/cimb46110780
- Varela L, van de Lest CHA, van Weeren PR, Wauben MHM. Synovial fluid extracellular vesicles as arthritis biomarkers: the added value of lipid-profiling and integrated omics. *Extracell Vesicles Circ Nucl Acids* 2024 ;5(2): 276–96. doi: 10.20517/evcna.2024.14
- Palacios A, Sampedro L, Sevilla IA, et al. Mycobacterium tuberculosis extracellular vesicle-associated lipoprotein LpqH as a potential biomarker to distinguish paratuberculosis infection or vaccination from tuberculosis infection. *BMC Vet Res* 2019; 15(1): 188. doi: 10.1186/s12917-019-1941-6
- Monari E, Troia R, Magna L, et al. Urine neutrophil gelatinase-associated lipocalin to diagnose and characterize acute kidney injury in dogs. *J Vet Intern Med* 2020; 34(1): 176–85. doi: 10.1111/jvim.15645
- Ugarte F, Santapau D, Gallardo V, et al. Urinary extracellular vesicles as a source of ngal for diabetic kidney disease evaluation in children and adolescents with type 1 diabetes mellitus. *Front Endocrinol (Lausanne)* 2021; 12: 654269. doi: 10.3389/fendo.2021.654269
- Cerón JJ, Ortín-Bustillo A, López-Martínez MJ, et al. S-100 Proteins: basics and applications as biomarkers in animals with special focus on calgranulins (S100A8, A9, and A12). *Biology (Basel)* 2023; 12(6): 881. doi: 10.3390/biology12060881
- Piibor J, Waldmann A, Prasadani M, et al. Investigation of uterine fluid extracellular vesicles' proteomic profiles provides novel diagnostic biomarkers of bovine endometritis. *Biomolecules* 2024; 14(6): 626. doi: 10.3390/biom14060626
- Yeo RW, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv Drug Deliv Rev* 2013; 65(3): 336–41. doi: 10.1016/j.addr.2012.07.001
- Amina SJ, Azam T, Dagher F, Guo B. A review on the use of extracellular vesicles for the delivery of drugs and biological therapeutics. *Expert Opin Drug Deliv* 2024; 21(1): 45-70. doi: 10.1080/17425247.2024.2305115
- Xiong Y, Lou P, Xu C, Han B, Liu J, Gao J. Emerging role of extracellular vesicles in veterinary practice: novel opportunities and potential challenges. *Front Vet Sci* 2024; 11: 1335107. doi: 10.3389/fvets.2024.1335107
- Kim HI, Park J, Zhu Y, Wang X, Han Y, Zhang D. Recent advances in extracellular vesicles for therapeutic cargo delivery. *Exp Mol Med* 2024; 56(4): 836–49. doi: 10.1038/s12276-024-01201-6
- Gervasi MG, Soler AJ, González-Fernández L, Alves MG, Oliveira PF, Martín-Hidalgo D. Extracellular Vesicles, the Road toward the Improvement of ART Outcomes. *Animals (Basel)* 2020; 10(11): 2171. doi: 10.3390/ani10112171
- Ba-Awadh HA, Olarinre IO, Alowaimer AN, Saadeldin IM, Swelum AA. Comparison between rapid and slow cryopreservation protocols for ram semen. *Slov Vet Res* 2023; 60: 249-57. doi: 10.26873/SVR-1586-2022
- Mogas T, García-Martínez T, Martínez-Rodero I. Methodological approaches in vitrification: enhancing viability of bovine oocytes and in vitro-produced embryos. *Reprod Domest Anim* 2024; 59 (Suppl. 3) : e14623. doi: 10.1111/rda.14623
- Pedrosa AC, Andrade Torres M, Vilela Alkmin D, et al. Spermatozoa and seminal plasma small extracellular vesicles miRNAs as biomarkers of boar semen cryotolerance. *Theriogenology* 2021; 174: 60–72. doi: 10.1016/j.theriogenology.2021.07.022
- Santos P, Almeida F. Exosome-based vaccines: history, current state, and clinical trials. *Front Immunol* 2021; 12: 711565. doi: 10.3389/fimmu.2021.711565
- Hoelzer K, Bielke L, Blake DP, et al. Vaccines as alternatives to antibiotics for food producing animals. Part 2: new approaches and potential solutions. *Vet Res* 2018; 49(1): 70. doi: 10.1186/s13567-018-0561-7
- El-Husseiny HM, Mady EA, Helal MAY, Tanaka R. the pivotal role of stem cells in veterinary regenerative medicine and tissue engineering. *Vet Sci* 2022; 9(11): 648. doi: 10.3390/vetsci9110648
- Pirisinu M. The long journey of extracellular vesicles towards global scientific acclamation. *Adv Pharm Bull* 2023; 13(3): 489–501. doi: 10.34172/apb.2023.049
- Koprivec S, Majdič G. Extracellular vesicles in domestic animals: cellular communication in health and disease. *Adv Exp Med Biol* 2024; 1450: 39-57. doi: 10.1007/5584\_2023\_779