ABSTRACT BOOK

UNLOCKING THE POTENTIAL OF EXTRACELLULAR VESICLES IN ONE-HEALTH, FOOD AND FEED

Annual Conference

24-25 th of May, 2024

Tartu, Estonia

Eesti Maaülikool
Estonian University of Life Sciences
Tartu Ülikool
Universitas Tartuensis
1632
COMBIVET
H-BOOST
Helsingin yliopisto
Helsingfors universitet
University of Helsinki
Universitas Studiorum Mediolanensis
BSEVs
Baltic Society of Extracellular Vesicles
Euroopa Liit
Euroopa
Regionaalarengu Fond
Eesti
tuleviku heaks

Estonian University of Life Sciences

PROCEEDINGS OF THE COMBIVET & OH-BOOST JOINT CONFERENCE 2024

24 – 25th of May, 2024

ISBN 978-9916-719-85-5

Publisher:
Estonian University of Life Sciences, Tartu, Estonia

Edited By:
Chanaka Premathilka
Gayandi Ekanayake
Dulmini N. Sapugahawatte
Suranga Kodithuwakku
Alireza Fazeli

Cover Design By:
Chanaka Premathilka
Amita Kulina

About the COMBIVET & OH-Boost Joint Conference 2024

This conference is jointly organised by the ERA Chair COMBIVET and OH-Boost Twinning Project of the Estonian University of Life Sciences in partnership with the University of Tartu (UT), University of Helsinki (UH) the University of Milan (UniMi) and Baltic Society of Extracellular Vesicles (BSEV). This conference marks the final gathering under the guidance of the ERA Chair COMBIVET. The COMBIVET and OH-Boost programs are supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 857418 COMBIVET and the European Union through Horizon coordination and support actions under grant agreement No. 101079349 OH-Boost respectively.

ACKNOWLEDGEMENT

COMBIVET
H-BOOST
TARTU ÜLIKOOL
UNIVERSITY OF TARTU
1632
www.emu.ee
Eesti Maaülikool
EMU
Estonian University of Life Sciences
UNIVERSITAS STUDIORUM MEDIOLANENSIS
BSEVs
BALTIC SOCIETY OF EXTRACELLULAR VESICLES
UNIVERSITY OF HELSINKI
European Union
European Regional
Development Fund
Investing
in your future

We acknowledge the support of our sponsors for the COMBIVET & OH-Boost Joint Conference 2024

PLATINUM

GOLD

SILVER

CONTENTS

PP 1.0 **Poster Presentations**

Welcome

I extend a warm welcome to all participants of the COMBIVET and OH-Boost Joint Conference 2024, the collaborative event organized by the COMBIVET ERA Chair of Comparative Medicine and the One Health-Boost (OH-Boost) in the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences along with our partners, University of Tartu, University of Helsinki and University of Milan, and the Baltic Society of Extracellular Vesicles. With great pride, I am excited to announce that this conference marks the 8th gathering focused on Extracellular Vesicles (EVs) organized by the Fazeli Lab.

In contemporary studies, the interest in EVs continues to grow across diverse scientific communities, particularly in the fields associated with cancer, infectious diseases, inflammation, and food science. Furthermore, EV plays a crucial role in intercellular interaction, EVs are considered to be the very attractive targets for theranostics. Whereas EVs were primarily investigated within the confines of human medicine, now it has expanded to the fields of veterinary medicine, and food science as well as in studies of plants and microorganisms. This evolutionary shift has turned EVs into an attractive and energetic domain for scientific studies.

Leading by COMBIVET ERA Chair of Comparative Medicine at the Institute of Veterinary Medicine and Animal Sciences of the Estonian University of Life Sciences, and previously under the ERA Chair of the Translational Genomics program (TransGeno) at the University of Tartu, we developed a robust collaboration between researchers with shared interest in studying EV, characterizing and investigate their potential to be used for understanding various human and animal diseases and conditions including infertility and other reproductive disorders, osteoarthritis, psoriasis and infectious diseases. Highlighting the ONE Health-Boost (OH-Boost) project which plays a crucial role in our collective

efforts is equally essential, particularly the contribution to enhance research and innovation excellence as well as strengthening management capacities at the Estonian University of Life Sciences, and the University of Tartu within the context of One Health.

As our journey has progressed, we have acquired substantial expertise across various facets of EV purification and characterization. These expertise and experiences have enabled us to publish our insights into the biology of EVs in esteemed journals, fostering robust collaborations and expanding our EV knowledge into emerging fields within life sciences and medicine. Through the organization of this conference, we aim to create a platform for sharing and integrating multidisciplinary scientific knowledge, as well as facilitating opportunities for networking, collaboration, and avenues for career advancement within the EV research community. In this 8th gathering dedicated to EVs, the conference theme is "Unlocking the Potential of Extracellular Vesicles: One Health, Food, and Feed," which emphasizes our commitment to exploring the multifaceted roles of EVs in promoting health across diverse domains.

I would like to express my heartfelt gratitude to all the speakers, participants, and members of the organizing committee for their invaluable contributions, which have been instrumental in ensuring the success of this conference. I sincerely hope that it fosters meaningful scientific debates and discussions.

I wish the COMBIVET & OH-Boost Joint Conference 2024 tremendous success. This conference marks the culmination of the COMBIVET project and serves as the final meeting of this successful initiative. Like other projects led by the Fazeli lab, COMBIVET has been a remarkable success, leaving a lasting legacy at the Estonian University of Life Sciences and within the Estonian scientific community. COMBIVET has significantly advanced research in comparative medicine and the One Health era.

I am particularly grateful to all my colleagues and coworkers who supported and contributed throughout this journey. The success of COMBIVET is a testament to the collaboration and expertise of the many individuals involved. I wish all of you a productive time, enriched by lectures, discussions, and the exchange of ideas over the next two days.

Best wishes for a rewarding and inspiring conference!

Alireza Fazeli
Professor
ERA Chair of Comparative Medicine
Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences
Tartu, Estonia

Organizing Committee

Event Planning and Logistics

Alireza Fazeli
Suranga Kodithuwakku
Gayandi Ekanayake
Dulmini N. Sapugahawatte
Chanaka Prematilaka
Omid Reza Mousavi
Subhashini Muhandiram
Kasun Godakumara
Getnet Midekessa
Signe Parts
Annika Hurt
Jennifer Raal
Amita Kulina
Urszula Malińska-Mark

Scientific Committee

Alireza Fazeli
Kristi Huik
Radko Avi
Mihkel Mäesaar
Suranga Kodithuwakku
Gayandi Ekanayake
Dulmini N. Sapugahawatte
Chanaka Prematilaka
Omid Reza Mousavi
Subhashini Muhandiram

Contacts

Annika Hurt
Project Assistant, ERA chair COMBIVET
Institute of Veterinary Medicine and Animal Sciences
Estonian University of Life Sciences
Tartu, Estonia
annika.hurt@emu.ee / combivet@emu.ee

DAY 1: 24th of May 2024

DAY 1: Featured Speakers (FS)

FS 1.1 | Antonio Marcilla

Área de Parasitología, Departamento Farmacia y Tecnología Farmacéutica y Parasitología, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de València, and Joing Research Unit on Endocrinology, Nutrition and Clinical Dietetics, Hospital Universitario y Politécnico "La Fe"-Universitat de Valencia, Spain

Biography

Dr. Antonio Marcilla is Full Professor of Parasitology and leads a research group that studies the role of extracellular vesicles in interkingdom interactions. He obtained his doctorate in Pharmacy (Department of Microbiology-Universitat de València, Spain) in 1991, working on the characterization of mannoproteins from the cell wall of the pathogenic fungus Candida albicans as a target for specific diagnosis, producing several monoclonal antibodies, some of them commercialized.

As a postdoctoral fellow at the National Institutes of Health (Bethesda, MD, USA) (1993-1995), he was involved in the characterization of protein-protein interactions in signal transduction in cancer cells, primarily basophils, monocytes. and macrophages. Subsequently, he returned to Valencia where he carried out two postdoctoral stays at the Institute of Cytological Research of Valencia (1995-1996) (currently the "Príncipe Felipe" Research Center), and the University of Valencia (1996-1998).

He began his teaching career as Assistant Professor of Parasitology, Faculty of Pharmacy-UV (1998-2002), Associate Professor (2002-2016). He obtained the position of Full Professor in 2016.

Since her appointment at the Department of Parasitology in 1998, he has worked in Molecular Parasitology, initially with Chagas' disease vectors (producing molecular tools to distinguish triatomine vectors), and later with parasitic trematodes, as well as with the nematode Strongyloides stercoralis. He has been involved in the analysis and molecular characterization of the host-helminth parasite interface, using "–omic" approaches (genomic, transcriptomic and proteomic), in order to identify new and specific targets for its control (diagnostic biomarkers, prognoses, treatment and/or vaccination).

In 2011 he began his research in extracellular vesicles as a source of these targets. His group described for the first time the presence of extracellular vesicles in parasitic helminths, as well as the presence of microRNAs in these vesicles. Supervisor of 11 Doctoral Theses and more than 20 Master's Theses, he has published more than 120 articles in high-impact scientific journals, with more than 22,265 citations (h-index: 45).

He has been a founding member of the Spanish Group on Extracellular Vesicles (GEIVEX) and also belongs to the International Society of Extracellular Vesicles (ISEV). Member of the editorial board of several prestigious journals in the field of parasitology and extracellular vesicles such as the Journal of Extracellular Vesicles (JEV). He is a Member of the Royal National Academy of Pharmacy (Spain).

FS 1.2 | Metka Lenassi

Associate Professor of Biochemistry and Molecular Biology and the head of the Laboratory for Extracellular Vesicle Research, at the Faculty of Medicine, University of Ljubljana, Slovenia

Biography

Her research work is focused on advancing the knowledge of extracellular vesicles (EVs) in cell-pathogen communication, using human-HIV interaction as a model system. Specifically, her group studies biogenesis, composition, targeting and functions of EVs released from cells expressing HIV protein Nef and their role in non-AIDS related conditions in HIV-infected individuals. Her group is also heavily involved in EV-biomarker studies and collaborate on this with several clinical partners. Their research focus is on optimizing biobanking of biological samples and methodology for EV isolation, and on evaluating EV- protein, RNA, and DNA components as disease state read-outs.

Metka is also dedicated to promoting EVs among wider research and medical community, through her involvement in teaching activities and EV-training programs, and through her roles in local (president of the Slovenian Network for Extracellular Vesicles, SiN-EV) and international (serving as the Executive Chair of Science & Meetings Committee of the International Society for Extracellular Vesicles, ISEV) EV societies.

FS 1.3 | Harinda Rajapaksha

Research Fellow, School of Molecular Sciences, La Trobe University, Melbourne, Australia

Biography

Harinda graduated from the University of Peradeniya, Sri Lanka, majoring in Agricultural Biology. Then he moved to Australia for higher studies, and he holds a PhD degree in Protein Biochemistry from the University of Adelaide, Australia. He has about 20 years of experience as a Bioinformatician and a Computational Chemistry researcher. Harinda also worked as a researcher at La Trobe University, Australia before moving to industry. In addition to being a researcher, he is an industry-seasoned data scientist and an accredited statistician with the Statistical Society of Australia. Currently, he is working as a data scientist at Oracle Corporation while engage in R & D in various bioinformatics applications. Other than his research involvements, Harinda is an aspiring musician holding qualifications in Indian classical music.

FS 1.4 | Janne Leivo

Principal Investigator, Adjunct Professor, University of Turku, Finland

Biography

Dr. Janne Leivo is an adjunct professor and the Early Cancer Detection group leader at the University of Turku, Finland. The group's primary focus is to design new concepts for early cancer detection and precision management with novel EV-based biomarkers and test technologies. Our research combines biomarker discovery, antibody and assay development, and clinical evaluation of biomarkers for the most prevalent solid cancers. Through clinical and industry collaborations, our activities are at the interface of basic, translational and clinical research, emphasizing diagnostic applications of EVs.

FS 1.5 | Karol Mierzejewski

Assistant Professor at the University of Warmia and Mazury in Olsztyn, Poland

Biography

Karol Mierzejewski received his PhD in the field of animal immunology and molecular biology at the University of Warmia and Mazury in Olsztyn (Poland). His doctoral thesis focused on the influence of PPAR-gamma ligands on the regulation of the immune response in the endometrium of pigs with LPS-induced inflammation. He has completed several internships, e.g. at the Department of Veterinary Medicine at the University of Milan, the Institute of Marine Research at the Spanish National Research Council in Vigo, and the Biomedical Research Center at the University of Vigo. He currently works as an assistant professor at the Department of Animal Anatomy and Physiology at the University of Warmia and Mazury in Olsztyn. The aim of his current research is to investigate the effects of polyethylene terephthalate (PET) microplastics (one of the most commonly used plastics in food packaging) on the content of extracellular vesicles (EVs) isolated from selected body fluids (blood, bile and urine), as well as the transcriptomic, proteomic and metabolomic profiles of different tissues (liver and kidney), which are the main source of EVs in bile and urine. In his research, he uses high-throughput methods such as RNA-Seq for transcriptome analysis and liquid chromatography/tandem mass spectrometry (LC-MS/MS) for proteome and metabolome analysis.

FS 1.6| Dulmini N. Sapugahawatte

Research Fellow, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Biography

Dulmini received her bachelor's degree with first class and Distinction from Mangalore University, India majoring in Microbiology in the year 2013. Then, she completed her master's degree in Medical Microbiology at the University of Peradeniya, Sri Lanka in 2016. Later, she completed her PhD in the year 2022 at the Department of Microbiology, Faculty of Medicine, the Chinese University of Hong Kong, Hong Kong SAR in Microbiology. Her doctoral research focus was "Characterization of Streptococcus agalactiae from humans and animals' and it involved the investigation of antimicrobial resistance, detection of possible zoonotic traits of GBS and application of novel MALDI-TOF-MS based typing method in collaboration with Swiss Tropical and Public Health Institute (Swiss TPH), Switzerland apart from genomics. After the doctoral training, she worked as a postdoctoral research fellow at the Chinese University of Hong Kong and dealt with several research works related to antimicrobial resistance, one-health and microbiome. She is currently a research fellow at EMU and working on antimicrobial properties of plant and animal-derived extracellular vesicles. Dulmini won the President's Award for Scientific Research in 2019 and the National Research Council (NRC) Merit Awards for Scientific Research in 2021 for her previous research work.

FS 1.7 | Marca Wauben

Professor in Cell Biology, Chair of Intercellular Communication
Division of Cell Biology, Metabolism & Cancer, Faculty of Veterinary Medicine, Utrecht University, Netherlands

Biography

Marca Wauben is a Full Professor of Cell Biology (Chair: Intercellular Communication) at the Department of Biomolecular Health Sciences, Faculty of Veterinary medicine, Utrecht University, The Netherlands. She is a pioneer in EV research and an expert in deciphering the role of nano-sized cell-derived vesicles (EVs) in intercellular communication in biological systems with emphasis on immunomodulation. Her research group has unveiled novel aspects in EV biogenesis, regulation of EV release, specific targeting, and cargo incorporation (including RNA, proteins, metabolites and lipids). By combining multi-omics with functional cellular modulation, the concept of EV-mediated modulation via the convergence of different EV-cargoes on nodes of regulation in cellular signal transduction pathways has been postulated. Milk-EV oriented research led to the development of tailored protocols for milk storage and milk EV isolation. Furthermore, integrated multi-omics and functional analysis of milk EV-mediated effects on T cells and epithelial cells, unveiled EV-cargo-function relationships and cross-species communication. Besides investigating fundamental immunological and regenerative aspects of EV-mediated communication, her research group works on EV-based biomarker discovery, (therapeutic) EV applications and the development of (nano)technology to analyze EVs, e.g. the pioneering work on high resolution flow cytometry-based technologies enabling single EV-based analysis and sorting.

Wauben is author of > n=150 peer reviewed scientific publications on EVs and (Co-)recipient of multiple EV-based consortium research grants, e.g. FP7 EU COST Action Microvesicles and Exosomes in Health and Disease (2012, vice-chair), H2020-MSCA-ITN-2016 TRAIN-EV research & training

Network (722148) (2017) and H2020-FETOPEN The EV Foundry (801367)(2018). She has been/and is involved in many national and global initiatives to build a scientific community centered around EV-biology; e.g. the founding of the International Society for Extracellular Vesicles (ISEV), the scientific journal "Journal of Extracellular Vesicles", the founding of the ISEV-ISAC-ISTH EV flowcytometry working group (www.evflowcytometry.org) and the founding of the Netherlands Society for Extracellular Vesicles (NLSEV) in 2018 (founding President). She is co-chair of the ISEV Milk EV Task Force and member of ISEV Regulatory affairs & clinical use of EV-based therapeutics Task Force. In 2019 she was awarded the ISEV special Achievement Award and in 2020 the ISEV life-time membership Award.

FS 1.8 | Alfredo Ambersone

Associate Professor in Plant Genetics and Genetic Diversity at the Department of Pharmacy, University of Salerno, Italy

Biography

Alfredo Ambrosone earned his PhD in Biotechnological Sciences from the University of Naples "Federico II" in 2008, focusing on plant tolerance mechanisms under abiotic stress. During the postdoctoral experience at the Cybernetics Institute (CNR) from 2010 to 2014, he explored nanobiotechnology, particularly the response to nanoparticle-induced stress and innovative strategies for gene silencing and signaling pathway control.

Prof. Ambrosone won a Marie Skłodowska-Curie Individual Fellowship under the HORIZON 2020- Excellent Science program at the University of Zaragoza, Spain, in 2015. Additionally, he has held visiting research positions at several prestigious institutions abroad, such as the University of Innsbruck, Ludwig Maximilians University of Munich, Phillips University of Marburg, and the Instituto de Nanociencia de Aragon.

His research now focuses on the genetic and molecular mechanisms affecting plant growth and secondary metabolite synthesis under environmental stresses. In recent years, he has investigated the potential of plant extracellular vesicles for both plant protection and human health. He is a founding member of the Italian Society for Extracellular Vesicles (EVITA).

He currently leads several national and international research projects, including as coordinator of a National Research Project (PRIN 2022) on plant extracellular vesicles and their role in plantmicrobiome interactions, and a Marie Skłodowska-Curie Postdoctoral Fellowship project (2023) on nanocarriers for RNA interference in plants.

Prof. Ambrosone has authored more 40 papers in leading journals in plant sciences and nanobiotechnology, and his scientific achievements include more than 50 contributions to national and international conference proceedings as poster and invited oral presentations. He served on the Board of Directors of the Italian Society of Agricultural Genetics from 2021 to 2023.

2.0. DAY 2: 25th of May 2024

DAY 2 Featured Speakers (FS)

FS 2.1| Gemma Chiva-Blanch

Associate Professor, Universitat Oberta de Catalunya (UOC), Barcelona; Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona; and Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Biography

PhD. in Medicine from the University of Barcelona (UB, Extraordinary Award 2012-213), Master in Food Development and Innovation (UB), Degree in Food Science and Technology (UB), and Diploma in Human Nutrition and Dietetics. Her research activity is focused on the search for new biomarkers of atherosclerosis, thrombosis and cardiovascular risk, and how certain dietary components influence cardiovascular risk through the modulation these biomarkers. She is a Fellow of the European Society of Cardiology (ESC), where she participates in different working groups. She has published more than 65 articles indexed in Pubmed, with more than 3500 citations (H-index 34).

FS 2.2| Madhusha Prasadini

PhD Student, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia

Biography

Madhusha Prasadani completed her BSc degree (Agriculture Technology and Management, majoring in Animal Science) in the year 2019 from the Faculty of Agriculture, University of Peradeniya, Sri Lanka. She continued her master's (MSc) degree in Biotechnology at the Postgraduate Institute of Agriculture, University of Peradeniya and completed it in 2021. She has worked as a graduate research assistant in the fields of environmental studies and oral cancer studies. She joined the COMBIVET ERA Chair team in March 2022 as a PhD student in Veterinary Medicine and Animal Science at the Estonian University of Life Sciences. She is currently working on identifying the potential of milk extracellular vesicles as a biomarker in the discovery of insulin resistance in dairy cattle and humans under the supervision of Prof. Alireza Fazeli, Prof. Hanno Jaakson and Prof. Vallo Volke.

FS 2.3| Suranga Kodithuwakku

Senior Research Fellow, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia and Professor in Animal Science, University of Peradeniya, Sri Lanka

Biography

Suranga graduated from the University of Peradeniya, Sri Lanka majoring in Animal Sciences with further training and conducting research at the Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan in Farm Animal Reproductive Biology. Later, he completed his PhD at the Department of Obstetrics and Gynaecology, Faculty of Medicine, The University of Hong Kong, Hong Kong in Reproductive Biology/Obstetrics and Gynaecology. In addition, he received further postdoctoral training in advanced molecular and cellular aspects of reproductive biology from MBL/University of Chicago, USA and the University of Hong Kong, Hong Kong SAR. Suranga's major research interests are molecular and cellular biological aspects of reproductive biology /physiology in humans and farm animals.

FS 2.4| Karen Forbse

Associate Professor of Molecular Endocrinology and Reproduction at the University of Leeds, UK

Biography

Dr Karen Forbes in an Associate Professor of Molecular Endocrinology and Reproduction at University of Leeds. Karen received her PhD in Physiology & Pharmacology from University Strathclyde. She then undertook a Postdoctoral Research Associate position in Reproductive Endocrinology at University of Manchester before being awarded a University Fellowship to start her own research team. In 2014 Karen moved to University of Leeds to take up a position as Lecturer in Molecular Genetics and she was promoted to Associate Professor in 2020.

Karen's research is focused on understanding how factors in the maternal environment (diet, microRNAs and extracellular vesicles) contribute to altered placental and fetal growth, particularly in pregnancies complicated by maternal diabetes. She has a programme of research spanning from understanding the basic mechanisms of disease progression, through to the development of potential novel therapeutics for prevention of these conditions.

FS 2.5 | Seppo Vainio

Professor, Developmental Biology Biochemistry and Molecular Medicine, University of Oulu, Finland

Biography

Seppo Vainio is a professor of developmental biology at the University of Oulu, Finland. He also serves as a scientific director of the Kvantum Institute at Oulu University, Finland. He is a team leader at Kvantum Institute and Infotech Oulu and A vice director GeneCellNano flagship. His team Is targeting the biology of cell-secreted extracellular vesicles (EVs) in nature and medicine. Vainio received his PhD in embryology at Helsinki University and completed his postdoctoral training at Harvard University in 1997. The goal of Dr. Vainio's lab was to identify the genes, signals and molecular pathways associated to urogenital system development. In the InfoTech Oulu, the focus area is novel biosensor strategies. He is particularly interested today in the cellular and organ-level Nano communication roles of EVs in organogenesis and in disease. Based on their developmental biology work and its relevance to evolution and disease he has formulated a meta-theory that integrates the epigenetic and the genetic aspects of EVs into a collective and testable framework for evolution and disease. Vainio is the author of around 200 scientific publications and is a member of the Oulu Cell-Matrix Research Center at Oulu University. Google Scholar papers (https://scholar.google.com/citations?user=p9fUP30AAAAJ&hl=en).

E-mail: Seppo.Vainio@oulu.fi.

FS 2.6 | Justus Reunanen

Academy Research Fellow, University of Oulu, Finland

Biography

Dr. Reunanen has worked as an Academy of Finland (AF) Postdoctoral Research Fellow in a three-year AF - funded research project "Molecular characterization of the human intestinal microbiota-host interactome" (2011-2014, 332 000 €). Next he received funding from AF to AF Research Fellow project "Fundamental molecular mechanisms behind microbial colonization of the human intestinal tract" (2016-2021, 909 000 €) at the Biocenter Oulu (BCO) and University of Oulu (UO), Finland, where he started as a PI on September 2016. In 2016 Dr. Reunanen received a 588 000 € funding from the BCO to conduct a four-year Spearhead project "Innovative approaches to discover novel antibiotics – metamining the human intestinal microbiota" (2016-2019). In 2018 Dr. Reunanen was nominated as a Spearhead project partner in BCO-funded Emerging project "Fetal microbiome and exosomes, developing immune system and health in children" (2018-2022) led by Prof. Terhi Tapiainen (MD, pediatrician). In 2019, Dr. Reunanen received a 416 000 € funding from the BCO for the Spearhead project "Secreted vesicles of the human intestinal microbiota in health and disease" (2020-2023). In 2023 Dr. Reunanen and Prof. Tapiainen received a 456 000 eur funding from the BCO to conduct the Spearhead project "Gut microbiome in obesity from birth to adulthood" (2024-2027). The research group of Dr. Reunanen has developed methodologies for extraction of EVs from multiple clinical sample sources and for the isolation of macromolecules in the EVs along with stream-lined in-house bioinformatics pipelines. The research group of Dr. Reunanen currently comprises of 1 postdoc and 5 PhD-students.

Abstracts of Featured Presentations

Day 01: 24th May 2024

AB 1.1: EVs in the host-helminth interaction and their usefulness in controlling diseases

Antonio Marcilla

Parasitic helminths are responsible for the most neglected diseases, which are associated and promote poverty. They affect over a billion people worldwide (WHO, 2015), and account for billions of cases every year. Worm species found in the phyla Nematoda and Platyhelminthes, are well recognized manipulators of host immune function and physiology.

Emerging evidence supports an important role for helminth-derived extracellular vesicles (EVs) in different processes like parasite's survival, favoring infection, immunomodulation and parasite adaptation to the host environment. Thus, these processes and highlights EVs as important mediators in host-parasite interactions as part of cross-kingdom communications.

Research on helminth extracellular vesicles (EVs) has increased in recent years and demonstrated that EVs shed by helminths could be explored as novel and specific control tools, as biomarkers of (asymptomatic) infections and disease prognosis after drug treatment, as well as targets for potential vaccines and novel treatments.

In our presentation evidence will be presented of the usefulness of helminth EVs as biomarkers, immune modulators and novel treatment strategies for different diseases.

AB 1.3: Cloud computing for life science research: specific to proteomics

Harinda Rajapaksha

Cloud computing has emerged as a transformative technology in life sciences, offering unprecedented opportunities for data storage, analysis, and collaboration. It is pivotal in revolutionising life science research across multiple domains, including genomics, proteomics, drug discovery, and bioinformatics.

Cloud-based platforms offer robust infrastructure and scalable resources well-suited for handling the massive volumes of biological data generated in life science research. These resources include scalable storage, on-demand computing, HPC, parallel and distributed computing, and workflow automation. By leveraging cloud infrastructure and services, researchers can accelerate their workflows, collaborate seamlessly across geographic boundaries, and access advanced computational resources without significant upfront investments.

Adopting cloud computing in life science research brings several challenges, such as data security, privacy concerns, regulatory compliance, and interoperability with on-premises infrastructure and research tools. However, the prospects of cloud computing in advancing life science research are promising, offering numerous opportunities for innovation.

Integrating artificial intelligence (AI) and machine learning (ML) algorithms with cloud-based platforms could revolutionise data analysis and decision-making in life science research. Streaming data processing frameworks and IoT devices could facilitate continuous monitoring of physiological parameters, disease outbreaks, and environmental factors, enabling proactive interventions.

In conclusion, by embracing cloud-based solutions, researchers can unlock new avenues for exploration, collaboration, and discovery in the quest to understand and improve human health and well-being.

AB 1.4: Extracellular vesicle glycosylations as biomarkers of cancer

Janne Leivo

For the past decade, the Early Cancer Diagnostics Group at the University of Turku has explored the use of carbohydrates as cancer biomarkers to overcome the limitations of the current immunoassays used in clinics. Throughout the disease progression, cellular metabolism adapts to the tumor microenvironment, which can result in alterations in the glycosylation of proteins and lipids. These aberrations of glycans have been known to be linked to tissue malignancy since the late 1940s and can be causally linked to all the hallmarks of cancer. We have found that by detecting the glycovariants (GV) of conventional cancer biomarkers, such as CA125, we can enhance the clinical performance of the immunoassays used for the diagnosis of different solid cancers. Intricately linked to the metabolism of cancer cells, the aberrant glycosylations on the surface of EVs can also be used as biomarkers. Currently, our EV research consists of three main focus areas: 1) Biomarker discovery with the use of synthetic antibody libraries and phage display. 2) Development and technical validation of the various analytical parameters of EV-immunoassays. 3) Clinical evaluation of EV-based biomarkers in collaboration with clinical partners. In the future, we aim to harness the full biomolecular complexity of EVs to detect the early pathological alterations of the host cell in a tissue/disease-specific manner.

AB 1.5: New insights of extracellular vesicles in the context of microplastic effect and distribution through organism

Karol Mierzejewski

Plastics have become ubiquitous in our daily lives, which has led to a widespread presence of microplastics (<5 mm) in the environment. With their increasing presence in water, food, and air, microplastic pollution has emerged as a pressing concern for public health and environmental sustainability. Recent research has highlighted the harmful effects of microplastics on organisms, so their mechanisms of action need to be further investigated.

Extracellular vesicles (EVs) are small membrane particles that contain proteins, lipids, RNAs and miRNAs and are secreted by almost all cell types. In the last decade, EVs have been identified as important mediators of communication with neighboring or distant cells. In addition, EVs eliminate toxic/unnecessary substances from the cell, suggesting that they play a role in maintaining intercellular homeostasis. In light of this, our study investigates the role of extracellular vesicles (EVs) in mediating the effects and distribution of polyethylene terephthalate (PET) microplastics, which are commonly found in food and beverages. We aimed to determine whether EVs facilitate the transport of PET particles and evaluate their impact on the global microRNA and metabolomic profiles of serum-derived EVs.

Our results show that serum-derived EVs indeed transport plastic particles and that PET microplastics induce changes in the miRNA and metabolome content of EVs. In particular, the identified differentially regulated miRNAs have effects on genes associated with prevalent lifestyle diseases, including cardiovascular disorders, metabolic syndromes, and carcinogenesis. This study significantly advances our understanding of how PET microplastics affect organisms via EV-mediated communication and sheds light on potential avenues for further research and strategic interventions.

AB 1.6: Innovative strategies utilizing extracellular vesicles to combat biofilms

Dulmini Nanayakkara Sapugahawatte

Bacterial biofilms in milking clusters, predominantly composed of pathogens like *Staphylococcus aureus*, *Streptococcus agalactiae* (GBS), and *Escherichia coli*, pose formidable challenges due to their resilient adherence to surfaces, and traditional disinfectant treatments often yield unsatisfactory outcomes. This persistence of bacterial biofilms in milking clusters often contributes to bovine mastitis, a global concern impacting animal health, milk quality, and potentially public health via zoonotic transmission risks. Recognizing the limitations of conventional control methods, this study investigates innovative approaches utilizing extracellular vesicles (EVs) derived from whey and algae spent media. Evaluation of whey EVs and algal-derived vesicles (algasomes) on established biofilms reveals promising inhibitory effects against all tested pathogens. Both, Whey and algae spent-derived EVs notably demonstrate strong inhibition across all strains. Statistical analysis confirms significant reductions in biofilm biomass compared to controls. These findings highlight the potential of whey EVs and algasomes as natural strategies for combating biofilms of mastitis-causing pathogens, offering avenues for reduced use of antibiotics and optimization of dairy and algae waste utilization.

AB 1.7: Deciphering the physiological role of extracellular vesicles in milk

Marca Wauben

In mammals, milk is the first functional food. Besides its nutritional value, milk contains bioactive structures composed of lipids, proteins, nucleic acids and metabolites that are involved in a kick-start for life. How these structures function is poorly understood. Recent discoveries highlighted the potential significance of milk extracellular vesicles (mEVs). mEVs are a heterogeneous population of lipid bilayer-enclosed particles released by cells for intercellular communication. Comparative mEV studies unveiled conserved miRNA and protein cargoes between species. Moreover, mEVs are stable and can cross epithelial borders suggesting that these EVs might be involved in inter-organism communication. We explored the molecular mechanisms of mEV-induced modulation of different cell types present in the gastrointestinal tract and found that mEVs stimulate the migration of epithelial cells and have immune-modulatory capacities, as shown by the inhibition of innate and adaptive immune responses. Integration of functional analyses and the mEV-proteome unveiled that mEV proteins converge on defined hotspots in canonical signal transduction pathways involved in these processes. Interestingly, a comparison of the T cell modulatory capacity of human mEVs derived from allergic or non-allergic mothers unveiled subtle differences in their modulatory activity and EV-cargoes. Moreover, we found that raw bovine mEVs could inhibit human T cell responses and that processing before mEV isolation could have detrimental effects on mEVs and their bioactivity.

These insights also show the potential for mEV-based applications, e.g., biomarkers, food enhancement and therapeutics. However, knowledge gaps regarding their mode-of-action, the impact of parental status and the preservation of mEV-functionality need to be addressed.

This work was supported by the Dutch Technology Foundation STW partnership program between Nutricia Research and Utrecht University; Friesland Campina Nederland B.V.; The EU Horizon 2020 Framework Programme: FETOPEN-801367 evFOUNDRY and Marie Skłodowska-Curie-722148 TRAIN-EV.

AB 1.8: Plant extracellular vesicles for natural product-driven approaches in nanomedicine

Alfredo Ambrosone

Plant extracellular vesicles (EVs) are promising non-mammalian alternative tools in nanomedicine. They may serve as natural drug delivery systems by efficiently transporting bioactive compounds into human cells. Moreover, bioengineering techniques could boost the ability of EVs to actively transport nucleic acids and target specific cells. However, the variability in the biomolecular composition of plant EVs, influenced by factors such as soil composition, phyto-treatments and fertilization regimes, as well as differences in genotypes, presents substantial challenges in standardizing their preparation. To address these challenges and facilitate bioengineering efforts, our research group has created plant biotechnological platforms that establish robust protocols for isolating and conducting thorough biophysical and multi-omics analyses of EVs from plant cell cultures and hairy roots (HRs) of diverse species, including both crops and medicinal plants.

Our studies have unveiled multiple health-promoting effects of EVs and other plant-derived nanovesicles. For instance, we have shown that HR-derived EVs from Salvia dominica can significantly inhibit the growth of pancreatic and breast cancer cells by promoting apoptosis. Furthermore, these HR-derived EVs have demonstrated considerable neuroprotective effects in a model of Parkinson's disease. Efforts to optimize these natural therapeutic properties through bioengineering are currently in progress. Collectively, these findings represent a significant breakthrough in harnessing plant extracellular vesicles for nanobiotechnology and eco-friendly approaches to nanomedicine.

Day 02: 25th May 2024

AB 2.1: Circulating EVs as biomarkers of cardiovascular disease

Gemma Chiva-Blanch

Atherothrombosis is the primary cause of death globally. This process is started and propagated by persistent damage to the vascular endothelium due to high cholesterol exposure, which results in endothelial activation and leukocyte recruitment, the development of endothelial dysfunction, the formation of atherosclerotic lesions and finally platelet aggregation and thrombosis leading to a major adverse cardiovascular event (MACE).

Circulating extracellular vesicles (EV) from leukocytes, platelets and endothelial cells are released at the bloodstream and, following local or systemic circulation, they transfer their molecular content to alter the phenotypic of recipient cells. In fact, EV actively contribute to the initiation, development, and progression of atherosclerosis as well as to thrombus formation, leading to MACE.

Blood cell and endothelial EV are elevated in subjects with cardiovascular risk factors or metabolic disorders and have been found to be predictive and diagnostic biomarkers for MACE. Several studies propose the application of cell-specific EV subpopulations as prognostic biomarkers for varying stages and severity of cardiovascular diseases (CVD). As a result, EV are starting to emerge as non-invasive surrogate biomarkers associated with unfavourable clinical outcomes in CVD. In addition, recent studies have shown that dietary patterns and some foods/food compounds may modulate EV release, suggesting a new mechanism explaining the relationship between diet and CVD.

AB 2.2: Deciphering the health status of dairy cows using milk-derived extracellular vesicles

Madhusha Prasadini

High-yielding dairy cows allocate more energy towards milk production and rely on their body reserves to a greater extent. This has led to an increased occurrence of metabolic diseases, such as insulin resistance (IR), due to prolonged periods of negative energy balance. Diagnosis of IR in cows currently involves invasive sampling, and advanced facilities and often detect the disease at severe stages. Therefore, it is essential to explore novel methods to overcome these problems. Extracellular vehicles (EV) are nano-sized particles released by all types of cells and involved in intercellular signalling and hold information regarding the health status of the secreting cells and thus serve as an ideal candidate for a biomarker. In addition, fatty acids detected in milk EVs have the potential to serve as promising agents for identifying the metabolic status of cows. Hence, conducting an in-depth analysis of fatty acids can help understand the health status of animals and detect early signs of IR.

AB 2.3: Exploring endocrine disrupting chemicals on female fertility through a holistic one health lens

Suranga Kodithuwakku

The dynamic interaction among humans, animals, and the environment has contributed to the complexity of many health issues, including infertility. Trends indicate a decline in fertility parameters among both humans and farm animals, with approximately 8 to 12% of couples worldwide experiencing infertility challenges. Consequently, there's been a surge in demand for assisted reproduction technologies (ARTs). Endocrine-disrupting chemicals (EDCs) have been linked to various health issues in humans, such as cancer, non-communicable diseases, and infertility, affecting the outcomes of ARTs as well.

Classifying the effects of EDCs on fertility solely under reproductive toxicology may not fully capture its impact, especially as it increasingly becomes a public health concern. The focus of EDC on female infertility stems from the intricate nature of female reproductive physiology. EDCs primarily enter the human body through food, water, pharmaceuticals, cosmetics, and air consumption. Furthermore, human activities, especially in agriculture, introduce new EDCs into the environment and food chains, with certain veterinary drugs and their residues now have been classified as EDCs. Therefore, adopting a One Health approach to understand the holistic impact of EDCs is crucial.

The primary challenges lie in identifying the chronic and transgenerational effects of EDCs, as data mainly come from small lab animal models (e.g., mice and rats) and short-term in vitro studies, which may not accurately reflect real-life scenarios. Addressing these challenges requires developing appropriate research models, identifying chronic effects of EDCs at physiologically relevant doses, discovering novel biomarkers for biomonitoring and early exposure detection, and implementing strategies to mitigate exposure. Therefore, examining the impact of EDCs on human

fertility from both One Health and comparative medicine perspectives is essential for progressing forward.

AB 2.4: Maternal EVs: key modulators of placental and fetal development in pregnancies complicated by maternal diabetes

Karen Forbes

With the global prevalence of diabetes increasing, more people of reproductive age are experiencing hyperglycaemic pregnancies. Diabetes in pregnancy is associated with maternal cardiovascular and metabolic complications. Pregnancies complicated by maternal diabetes also increase the risk of short and long-term health complications for the offspring, including increased fetal growth and elevated risk of developing cardiometabolic disease throughout life. Despite advanced methods for improving maternal glucose control, the prevalence of adverse outcomes associated with maternal diabetes remains high.

In our lab, we have used blood and placental samples collected from women with diabetes in pregnancy; novel in-vitro and ex-vivo models of human placenta; and in-vivo models of pregnancy in mice, to begin to unravel the complex link between maternal diabetes and adverse pregnancy outcomes.

In this talk, I will present data that demonstrates that maternally-derived extracellular vesicles (EVs) and their microRNA (miRNA) cargo are key factors linking maternal diabetes to offspring health. I will then discuss potential avenues for therapeutic intervention that could alleviate the short-and long-term complications associated with maternal diabetes.

AB 2.5: Building value from the biological nanoscale to the macroscale

Seppo Vainio

The discovery that cell-secreted vesicles (EVs) provide a universal communication system set many relevant challenges for the experimental sciences. First as the EVs become loaded with various types of cellular factors and are become surrounded by a corona these set challenged to their biochemical, molecular and functional analytics. Traditionally experimental biology and especially functional genetic studies have been targeted at the single molecular level such as those of the secreted signals, their receptors and signal transduction components. The players here have been characterized one by one via targeted methods such as the loss and the gain of function studies. The EVs represent a very new mode of signalling mechanism in nature. Indeed, wealth of EVs that have been constructed by various combinations of the same molecules that are present inside the cells are loaded to the EVs and secreted. Thus, technologies to target such complexity in more specific ways are needed. The other major issue is the need for mapping the origin and cargo of EVs with in tissues, organs and organisms including the transcytosis to cross biological barriers. Moreover, the EVs seem to mediate cross species and even interkingdom communication. All the openings set exiting question for biology, medicine, evolutionary biology and call for technological solutions. My team has targeted EVs in the context of the Finnish nature, its berries and the Forrest. We have studied EV changes to physical stimuli. We have engineered the functions and cargo of EVs aiming to address their roles in homeostasis and diseases. Moreover, the EV signalling system is functional in mammalian kidney organogenesis. Challenges remain to target their specific roles in coordination of ontogenesis. Data in these research lines will be presented and discussed.

Oral presentations

Day 01: 24th May 2024

OP 1.1: Fecal EVs: emerging as a bioindicator of gut physiology and pathophysiology

Chanaka Premathilaka

Diagnosis, prognosis, and monitoring of disease and health conditions in farm animals based on an easily accessible, non-invasive sampling method is a highly attractive prospect for farmers and veterinarians. Recently, faecal extracellular vesicles (fEVs), have emerged as promising entities for elucidating gut physiology and pathophysiology, as well as for identifying disease-related biomarkers. However, there have been no reports up to date on enriching EVs from ruminant faeces and their application in disease diagnosis, prognosis, and monitoring. In our study, we were able to devise a protocol for the enrichment of fEVs from cattle faeces with minimal contaminants and demonstrated the presence of protein, DNA and RNA cargo in fEVS. EVs isolated from faeces of Cryptosporidium infected animals are significantly smaller and less negative in zeta potential compared to EVs isolated from faeces of healthy animals. This observation underscores those pathogenic infections significantly influence the general characteristics of fEVs. These fEVs can be used with available assay platforms to detect early biomarkers and also can be used to understand microbial diversity, dysbiosis, or even the origin of the EVs with additional investigations. The study demonstrates that EVs isolated from faeces can be used as a non-invasive tool for the diagnosis and monitoring of gut-related pathogens and host-pathogen interactions.

OP 1.2: Plant extracellular vesicles utilization in food safety and hygiene

Gayandi Ekanayake

In the evolving landscape of food safety and hygiene, Plant Extracellular Vesicles (EVs) are carving a niche for themselves. These nano-sized vesicles, secreted by plant cells, are packed with proteins, lipids, and nucleic acids, playing a pivotal role in plant defence mechanisms and intercellular communication. The potential of these EVs in food safety, particularly their antimicrobial activity, is a burgeoning area of research.

Our focus narrows down to the specifics of potato peel-derived EVs. Conventionally discarded as waste, potato peels are now being recognized as rich sources of EVs. These EVs have demonstrated promising results against *Bacillus cereus*, a common foodborne pathogen. The antimicrobial properties of potato peel-derived EVs could revolutionize food safety measures, offering innovative, eco-friendly, and cost-effective solutions. This talk will explore the untapped potential of plant EVs in transforming waste into a valuable resource, thereby contributing to a safer and healthier food supply chain.

Day 02: 25th May 2024

OP 2.1: Role of extracellular vesicles in stress-induced interactions between embryo and maternal cells

Seyed Omid Reza Mousavi

This presentation will focus on the crucial role of extracellular vesicles (EVs) in facilitating communication between maternal cells and the embryo at the cellular level. Specifically, we explore the hypothesis that endometrial stress may influence trophoblast transcriptome by transmitting stress signals via EVs. Using an in vitro model, we subjected receptive endometrial cells (RL95-2) to stress induced by CoCl2 and enriched EVs from the conditioned media. These EVs were then administered to JAr trophoblast spheroids, mimicking the embryo, for further analysis. Our findings revealed through sequencing, demonstrate significant alterations in EV miRNA and transcriptomic profiles in response to stress. Notably, stressed EVs were found to modulate gene expression in trophoblast cells. Our study unravels the intricate mechanisms by which EVs mediate stress signals from mother to embryo, shedding light on potential implications for implantation failure.

Poster presentations

PP 1.1: Extracellular vesicles from lamb faeces: a potential indicator of gut physiology and pathophysiology

Chanaka Prematilaka[1], Suranga Kodithuwakku[1,2], Toomas Orro[1], Aneta Andronowska[3], Alireza Fazeli[1,4,5]

[1] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia. [2] Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka. [3] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland. [4] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, Tartu University, Tartu, Estonia. [5] Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.

Gut health is linked with the development and production of farm animals. Thus, studying gut physiology and pathology is receiving more attention. Faecal extracellular vesicles (fEVs) have emerged as a promising tool for monitoring gut health, and disease diagnosis. It is hypothesized that the EVs secreted by pathogens could play a crucial role in mediating pathogenic effects in the digestive tract. Diagnostic tools developed from fEVs present a non-invasive approach to disease diagnosis and monitoring. This study aimed to optimize a method for fEV enrichment from lamb faeces and characterize them following the guidelines from the International Society for Extracellular Vesicles (ISEV 2023). Faecal samples were directly collected from the rectum of the 17-day-old lambs (n=5). Samples containing 0.3 g of faeces were suspended in PBS, sequentially centrifuged to remove debris, and subjected to size exclusion chromatography to enrich fEVs. Twenty fractions were collected from size exclusion chromatography (SEC). Enriched particles were characterized using nanoparticle tracking analysis (NTA), protein quantity assay, and

transmission electron microscopy (TEM). NTA analysis showed enrichment of particles in fractions 5 to 9. The total particle concentration was $7.5 \pm 1.93 \times 10^{10}$ particles/mL. The size of nanoparticles lay in the range from 50 nm to 300 nm and the average particle size was 157.82 ± 3.56 nm. TEM results revealed the presence of typical cup-shaped EV like particles. Furthermore, the protein assay results illustrated that the fEV-enriched fractions had minimal to no soluble protein contaminations. Overall results show that fEVs of sufficient purity can be enriched from lamb faeces using the described protocol. Further research is required to investigate the utility of these fEVs as a non-invasive tool for monitoring and diagnosing gut health.

PP 1.2: Innovative strategies utilizing extracellular vesicles from whey and algae spent media to combat biofilms of bovine mastitis pathogens

Dulmini Nanayakkara Sapugahawatte[1], Kasun Godakumara[1], Mihkel Mäesaar[2], Gayandi Ekanayake[1], Deep Bhattacharya[1], Getnet Balcha Midekessa[1,3], Suranga Kodithuwakku[1,4], Mati Roasto[2], Genu Geetha Bai[5], Timo Kikas[5], Aneta Andronowska[6], Alireza Fazeli[1,2,7]

[1]Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia. [2] Chair of Veterinary Biomedicine and Food Hygiene, Estonian University of Life Sciences, Tartu, Estonia. [3] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia. [4] Department of Animal Sciences, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka. [5] Chair of Biosystems Engineering, Institute of Forestry and Engineering, Estonian University of Life Sciences, Tartu, Estonia. [6] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland. [7] Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Sheffield, United Kingdom.

Bovine mastitis, a global dairy cattle disease, impacts animal health, milk quality and public health due to its zoonotic potential. *Staphylococcus aureus*, *Streptococcus agalactiae* (GBS), and *Escherichia coli* are the key mastitis-causing pathogens. This study examined whey and algae spent media-derived extracellular vesicles (EVs) effects on in-vitro biofilm formation. EVs were enriched utilizing pasteurized milk's whey (Whey EVS) and *Chlorella vulgaris* algae spent medium (algasomes). *S. aureus* ATCC 25923, *S. agalactiae* ATCC BAA 116, and *E. coli* ATCC 53868 were standardised to McFarland standard 1.0 and cultivated in 96-well microtitre plates for 24 hours at 35°C to establish the biofilm in wells. Following incubation, plates were washed and exposed to whey EVs and algasomes ($1x10^9$ particles/mL, 100µL), followed by further incubation at 35°C for 24 hours. Biofilm biomass was evaluated through crystal violet staining, under an optical density of 620 nm and compared with PBS-

negative controls. Gentamicin at 10μg/mL served as the positive control. Whey EVs exhibited inhibitory effects on the established biofilms of *S. aureus*, GBS, and *E. coli* by 9.4%, 6.5%, and 7.4%, respectively. In contrast, algasomes showed inhibition of 16.1%, 9.66%, and 8.88% for the same bacterial strains compared to the negative control. Statistical analysis revealed significant inhibition of biofilm growth by whey EVs against *S. aureus* (p=0.002), GBS (p=0.023), and *E. coli* (p=0.017) compared to PBS. Similarly, algasomes significantly inhibited *S. aureus* (p=0.016) and GBS (p=0.012), although failed to inhibit *E. coli* biofilms effectively. Importantly the inhibition of *S. aureus* and GBS biofilms by algasomes, as well as GBS biofilm inhibition by whey EVs, were significantly lower compared to the inhibition observed with the positive control. This study shows whey EVs and algasomes can effectively inhibit further growth of established biofilm, offering natural and innovative strategies to combat mastitis-causing pathogens. Further investigation into their components and mechanisms could reduce antibiotic usage and optimise dairy and algae waste utilisation.

PP 1.3: Simultaneous detection of sigma-1 receptor and tetraspanins in live-cells and extracellular vesicles

Edijs Vavers[1], Sergei Kopanchuk[1], Santa Veiksina[1], Chaitanya Mathur[2], Ago Rinken1[1]

[1] University of Tartu, Faculty of Science and Technology, Institute of Chemistry, Ravila 14A, 50411 Tartu, Estonia. [2] University of Tartu, Faculty of Science and Technology, Institute of Technology, Nooruse 1, 50411 Tartu, Estonia.

Sigma-1 receptor (Sig1R) is an endoplasmic reticulum (ER) membrane protein which has been shown to modulate neuronal extracellular vesicle (EV) release. There have been identified several potential direct interactions of Sig1R with proteins involved in vesicle generation and transport. Notably, Sig1R has been found to be enriched in plasma exosomes which were isolated from individuals diagnosed with major depressive disorder. However, the characteristics and exact role of Sig1R containing EVs remain unclear.

We have generated and expressed fluorescent protein tagged Sig1R together with tetraspanins as markers of EVs for live cell imaging and characterization of isolated EVs. The MultiBacMam system was used for simultaneous co-expression of Sig1R-mCherry with EGFP-CD9 and Sig1R-EYFP with either CD63-mCherry or CD81-miRFP670. Time-lapse microscopy was used to evaluate localization and dynamics of expressed proteins in live cells. Tangential flow filtration was applied for concentration and isolation of EVs from conditioned SK-OV-3 cell culture media. The multi-well total internal reflection fluorescence imaging assay system was used for characterization of Sig1R containing EVs. Sig1R was found in nuclear envelope, ER and spot-like structures. In the cells, tetraspanins CD9 and CD81 labelled vesicles, plasma membrane and cell protrusions, while CD63 demonstrated weaker plasma membrane and

stronger intracellular vesicle labeling associated with multivesicular bodies. Fluorescent protein tagged Sig1R and tetraspanins were detected in extracellular space and in isolated EVs. We observed poor colocalization between Sig1R with tetraspanins CD9 and CD81. While higher interaction of Sig1R with CD63 was detected, the colocalization was cell state and condition dependent.

Limited colocalization with plasma membrane targeted tetraspanins and higher interaction with intracellular CD63 could indicate that Sig1R might be exocytosed from intracellular vesicles of endosomal origin. Thus, Sig1R can serve as a novel marker of exosomes that warrants further investigation for potential applications.

PP 1.4: Utilizing potato peel-derived extracellular vesicles: a new approach to combat *Bacillus cereus* ATCC 11778

Gayandi Ekanayake[1], Dulmini Nanayakkara Sapugahawatte[1], Kasun Godakumara[1], Getnet Balcha Midekessa[1,2], Mati Roasto[3], Aneta Andronowska[4], Rajeev Bhat[5], Alireza Fazeli[1,2,6]

[1] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia. [2] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila St. 14b, 50411 Tartu, Estonia. [3] Chair of Veterinary Biomedicine and Food Hygiene, Estonian University of Life Sciences, Kreutzwaldi 56/3, 51006 Tartu, Estonia. [4] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland. [5] ERA-Chair for Food (By-) Products Valorisation Technologies (VALORTECH), Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia. [6] Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Sheffield, United Kingdom.

Extracellular vesicles (EVs) have emerged as a potent means of intercellular communication in recent years. Being membrane-bound biological nanoparticles, EVs are capable of transporting labile biochemicals between cells affecting the physiological and pathological conditions of target cells. EVs are observed in all kingdoms, including plants. In our investigations into the proteome of potato peel apoplastic fluid, we have observed that they produce EVs enriched with proteins that take part in the plant's defence mechanism in both biotic and abiotic stress scenarios. We have made further investigations into the functionality of potato peel-derived EVs by evaluating their efficacy against common foodborne pathogens such as *Bacillus cereus*. Our experiments show that potato peel-derived EVs are capable of inhibiting the growth of *B. cereus* (ATCC 11778) by 55.01±0.07% in moderate doses (1x 10^9 particles/ml), an enriched functionality compared to the 32±0.18% growth inhibition that

can be achieved using unpurified potato peel (crude) apoplastic fluid. Data are presented as mean ± standard error of the mean (SEM), (n=3). The carrying capacity and the doubling time of the bacteria were significantly affected by the actions of potato peel-derived EVs. To elucidate the possible methods of the observed bacterial growth inhibition, we have measured the antibiofilm effects of potato peel-derived EVs on planktonic and preformed biofilm phases. Potato peel-derived EVs were able to significantly reduce the formation of biofilms (27.59±0.002%, p=2.30e-04) compared to the crude and eradicate the preformed biofilms (18.55 ±0.002 %) by *B. cereus* indicating a robust antibiofilm capability. These results were the conclusion of three independent biological replicates. Mentioned results show that potato peel-derived EVs are antimicrobial agents with immense potential in the food hygiene industry. Even though Proteomic studies confirmed some of the proteins involved in the antimicrobial effect, the overall mechanism of EV-based bacterial growth inhibition is not completely understood. With further development, these EVs can be used as food preservatives and antimicrobial agents in food packaging.

PP 1.5: Cow milk-derived extracellular vesicles can reflect the metabolic status of lactating cows

Madhusha Prasadani[1], Priit Karis[2], Merike Henno[2], Sirje Kuusik[2], Aneta Andronowska[3], Suranga Kodithuwakku[1,4], Hanno Jaakson[2], Vallo Volke[5], Alireza Fazeli[1,5,6]

[1] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Estonia. [2] Chair of Animal Nutrition, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Estonia. [3] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Poland. [4] Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka. [5] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Estonia. [6] Division of Clinical Medicine, School of Medicine and Population Health, University of Sheffield, Sheffield, United Kingdom.

High-yielding dairy cows are more prone to negative energy balance, leading to an increased incidence of metabolic disorders such as insulin resistance (IR). Detecting IR in cows involves invasive sampling and complex procedures. Therefore, it is imperative to discover novel methods and biomarkers to improve IR diagnosis. Extracellular vehicles (EV) involved in intercellular signaling are being investigated to determine the pathophysiology of diseases and the development of diagnostic methods. Therefore, the present study aimed to study the enrichment of bovine milk EV (MEV) and Glucose Tolerance Test (GTT) to detect IR status using the area under the curve for Insulin. Physicochemical analysis of MEV revealed that particle diameter was significantly higher ($p \leq 0.05$) in the high IR group compared to low and moderate IR groups. There were no differences in particle concentrations of milk samples obtained from the high IR group compared to the low and moderate IR groups. Fatty acids (FAs) analysis resulted in 72 different FAs in milk samples and 26 FAs in MEVs while 6 and 3 long-chain FAs were significantly abundant ($p \leq 0.05$)

in high IR animal milk and MEVs samples respectively, indicating their adipose tissue origin. Thus, the MEV-FA profile may have the potential to serve as promising bioindicators of cow metabolic disease conditions warranting in development of IR-specific, non-invasive, early-stage biomarkers.

PP 1.6. The Impact of extracellular vesicles from commercially available milk on *Staphylococcus aureus* growth

Deep Bhattacharya[1], *Dulmini Nanayakkara Sapugahawatte*[1], *Getnet Balcha Midekessa*[1,2] *Suranga Kodithuwakku*[1,3], *Mati Roasto*[4], *Gayandi Ekanayake*[1] *and Alireza Fazeli*[1,2,5]

[1] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51006 Tartu, Estonia. [2] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 14b, 50411 Tartu, Estonia. [3] Department of Animal Sciences, Faculty of Agriculture, University of Peradeniya, 20400 Peradeniya, Sri Lanka. [4] Chair of Veterinary Biomedicine and Food Hygiene, Estonian University of Life Sciences, Kreutzwaldi 52, 51006 Tartu, Estonia. [5] Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.

The increasing prevalence of antibiotic resistance in *Staphylococcus aureus* poses a significant threat to both animal and human health. Milk, recognized for its potent antibacterial components that enhance offspring immunity, serves as a promising avenue for combating this challenge. Extracellular vesicles (EVs), which facilitate intercellular communication, offer the potential for targeted antimicrobial delivery and insights into host-pathogen interactions. With a lipid bilayer structure and encapsulation of various biomolecules including proteins, RNAs, and DNAs, EVs present a rich area for investigation. The following research aimed to isolate EVs from pasteurized and unpasteurized milk whey (industry byproduct) by Tangential Flow Filtration (TFF) system and check the effect of EVs on the growth of *S. aureus* ATCC 25923. The bacteria were co-cultured with milk-derived EVs, namely pasteurized (P), concentrated pasteurized (CP) and

unpasteurized (UP) industry whey at $1x10^7$, $1x10^6$, and $1x10^5$ Colony Forming Units per millilitre (CFU/ml). Bacterial growth was tracked by measuring absorbance at 620 nm over 24 hours at 37°C. Phosphate buffer solution (PBS) with bacteria served as the negative control and all analyses were conducted in triplicate at the biological level, with each biological replicate comprising three technical replicates. The successful enrichment of EVs from various fractions of industrial whey was achieved, with their size and morphology aligning with previously reported characters of TFF-derived milk EVs. Notably, these EVs exhibited the capability to impede the growth of *S. aureus* ATCC 25923. Although the observed inhibition of *S. aureus* growth was statistically insignificant (P=0.02, Student's t-test), confirmation using bacterial growth inhibition through CFU reduction assay is essential. This study highlights the significance of industrial whey as a vital source of EVs, providing a sustainable solution for waste repurposing with inherent antimicrobial properties. These findings highlight a promising avenue for continued exploration in this burgeoning field.

PP 1.7. Effect of donor cell microenvironment in paracrine function of extracellular vesicles

Norhayati Liaqat Ali Khan[1,2], Subhashini Muhandiram[3], Keerthie Dissanayake[3,4], Kasun Godakumara[3,4], Getnet Midekessa[3,4], Aneta Andronowska[5], Paul R. Heath[1], Suranga Kodithuwakku[3,6], Amber Rose Hart[1], Alireza Fazeli[1,3,4]

[1] Division of Clinical Medicine, School of Medicine and Population Health, The Medical School, University of Sheffield, Sheffield, UK. [2] Centre of Preclinical Science Studies, Faculty of Dentistry, University Teknologi MARA (UiTM), Malaysia. [3] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Estonia. [4] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, Tartu, Estonia. [5] Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland. [6] Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

The emerging role of extracellular vesicles (EVs) in embryo-maternal communication has gained considerable interest in their therapeutic potential within assisted reproductive technology, particularly in enhancing implantation success. The paracrine function of EVs is central to their therapeutic potential. We previously showed that increased milk fat globule protein-8 (MFGE8) secretion from endometrial cells in response to trophoblast EVs can be used as an indicator of EV mediated embryo-maternal communication. In this study, we investigated how the microenvironment of trophoblast cells influences the generation of EVs and their subsequent paracrine effects, using MFGE8 secretion as an indicator. The trophoblast analogue human chorionic carcinoma cell line (JAr) was cultured using two different methods: conventional monolayer culture (2D) and suspension culture as spheroids (3D). We investigated how the cellular

growth environment influences the physical, biochemical, and cellular signaling characteristics of the EVs produced by these cells. The 2D and 3D cell culture-derived EVs did not show significant differences in term of their size or morphology. However, 3D EVs produced more EVs compared to 2D EVs. The proteomic cargo profile of 3D EVs was substantially different from 2D EVs. The paracrine signaling potency, as measured in MFGE8 secretion, showed that 2D EVs were more potent in cellular signaling than 3D EVs. The bioactive substances secreted by trophoblast cells change depending on the cellular microenvironment and affect their paracrine signaling.

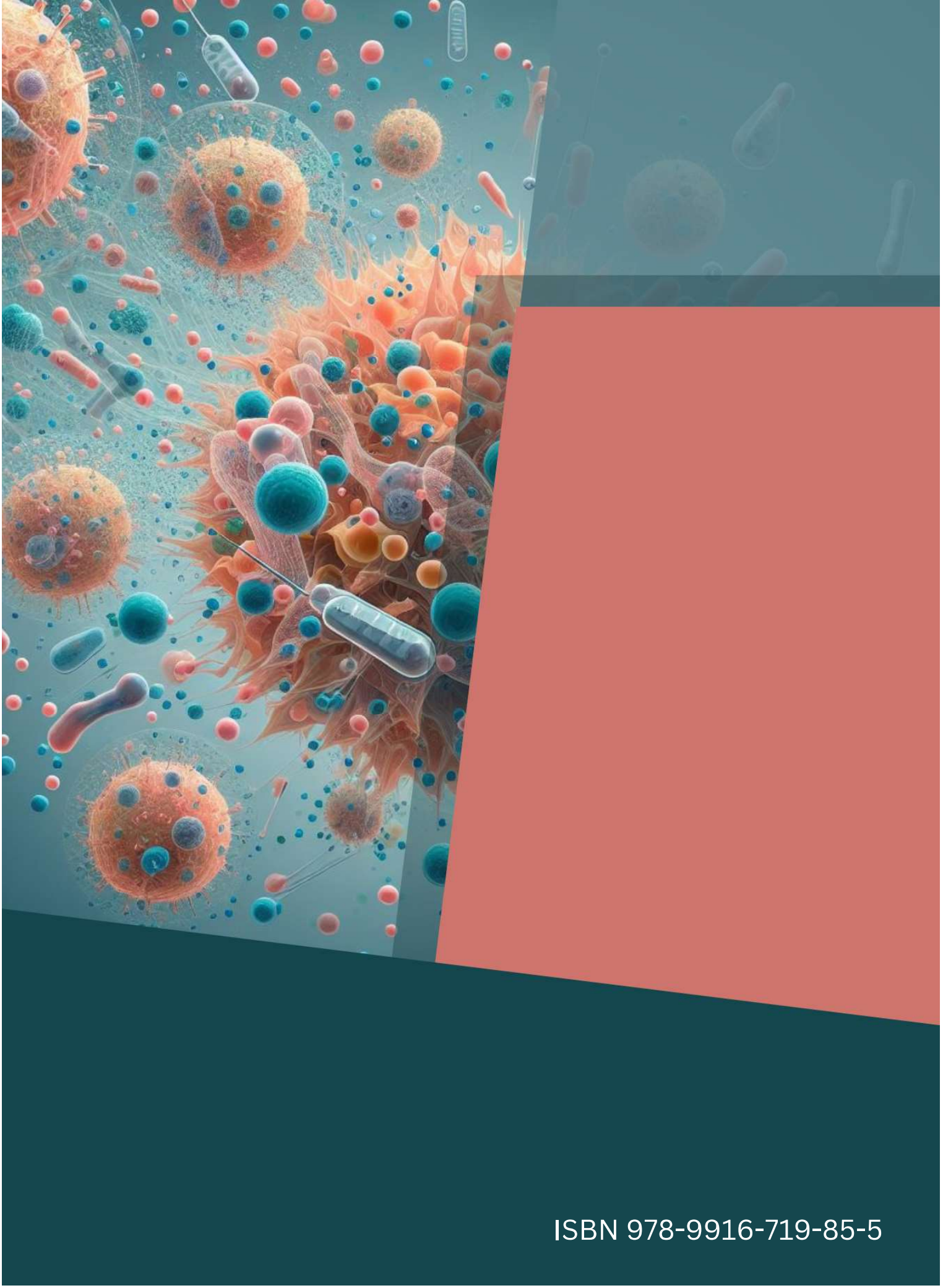
PP 1.8: Role of extracellular vesicles in stress-induced interactions between embryo and maternal cells

Seyed Omid Reza Mousavi[1], Qurat Ul Ain Reshi[1,2], Kasun Godakumara[1], Suranga Kodithuwakku[1,3], Alireza Fazeli[1,2,4]

[1] Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, 51006, Tartu, Estonia. [2] Department of Pathophysiology, Institute of Biomedicine and Translational Medicine, Faculty of Medicine, University of Tartu, 14B Ravila, 50411 Tartu, Estonia. [3] Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka. [4] Division of Clinical Medicine, School of Medicine & Population Health, University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK.

Extracellular vesicles (EVs) play a crucial role in mediating communication between the embryo and maternal cells during early pregnancy. We hypothesized that endometrial stress could be implicated in embryo development and functions via transmitting stress signals via EVs. Therefore, his study was designed to investigate the role of transmitting stress signals via EV miRNA from maternal cells to the trophoblast cells during the preimplantation period. RL95-2 cells, an in vitro model of receptive endometrial cells, were exposed to stress using 200 μM of CoCl2 for 24 hours and EVs were enriched from the conditioned media. JAr trophoblast spheroids, mimicking the embryo, were treated with EVs derived from stressed and unstressed RL95-2 cells for 24 hours. Next-generation sequencing (RNAseq) was used to analyse EV miRNA and transcriptomic changes in treated and untreated JAr spheroids. Analysis revealed significant changes in 25 miRNAs between stressed and unstressed EVs. Transcriptomic analysis identified 6 and 27 differentially expressed mRNA in JAr spheroids treated with stressed cell-derived EVs compared to unstressed EVs and the control group, respectively. Reactome pathway enrichment analysis indicated that stressed EVs can alter gene

expression in trophoblast cells, partly through EV miRNA. Overall, our findings suggest that EVs play a role in transmitting stress signals from mother to embryo.



ISBN 978-9916-719-85-5

Eesti Maaülikool
Estonian University of Life Sciences

TARTU ÜLIKOOL
UNIVERSITAS TARTUENSIS
1632

COMBIVET

H-BOOST

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

BSEVs
BALTIC SOCIETY OF EXTRACELLULAR VESICLES

Euroopa Liit
Euroopa
Regionaalarengu Fond

Eesti
tuleviku heaks