I INTERNATIONAL WORKSHOP 2021

ON APPLIED CELLULAR AND MOLECULAR BIOLOGY

Abstract's book

International Workshop on Applied Cellular and Molecular Biology

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DOCTORADO EN CIENCIAS MENCIÓN
BIOLOGÍA CELULAR Y MOLECULAR
APLICADA

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BIOLOGÍA CELULAR Y MOLECULAR
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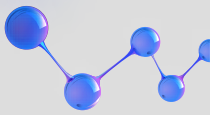
I INTERNATIONAL WORKSHOP 2021
ON APPLIED CELLULAR AND MOLECULAR BIOLOGY

November
25th - 26th

STREAMING BY zoom



Workshop Programme

Day 1 — Thursday, 25 November 2021 — Morning

09:00 - 09:15 — **Welcoming and Opening Ceremony**

Institutional Welcoming

Dr. Rodrigo Navia Diez, Vicerrector for Research and Postgraduate Studies, VRIP, Universidad de La Frontera, Temuco, Chile.
Dr. Marjorie Reyes Díaz, Organizing Committee, Universidad de La Frontera, Temuco, Chile.

Opening Conference

09:15 - 09:50 — **Dr. Vinicius Maracaja-Coutinho, Universidad de Chile, Chile**
Solving real-life problems through Genomics and Bioinformatics.

Symposium I. Biotechnology of Bioresources
Co-chairs: Dr. Marjorie Reyes Díaz & Dr. León Bravo Ramírez

09:50 - 10:25 — **Dr. Adriano Nunes Nesi, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Brazil.**
The Role of Mitochondrial Metabolism in Plant Stress Responses.

10:25 - 10:50 — **Dr. Laura Bertini, Department of Ecological and Biological Sciences, University of Tuscia, Italy.**
Small temperature changes act as drivers for metabolic reprogramming of *C. quitensis* plant and associated microbes in the Antarctic scenario.

10:50 - 11:15 — **Dr. Carolina Padovan, Department of Microbiology and Immunology, Federal University of Alfenas, Brazil.**
Implications of adhesin discovery in the emergent yeast pathogen *Trichosporon asahii.*

11:15 - 11:25 — **Break**

Symposium I. Biotechnology of Bioresources
Co-chairs: Dr. Cledir Rodrigues-Santos & Dr. Marysol Alvear Zamora

11:25 - 11:45 — **Dr. Manoel Oliveira, Minister of Health, Brazil**
Molecular diagnosis of sporotrichosis: What methodologies are applied for the identification of species in our Research Group?.

11:45 - 12:05 — **Dr. Cristian Paz Robles, Departamento de Ciencias Básicas, Universidad de La Frontera, Temuco, Chile.**
Natural and semisynthetic bioactive drimane sesquiterpenoids isolated from *Drimys winteri.*

12:05 - 12:25 — **Dr. Claudia Sanhueza, Centro de Medicina Traslacional, Universidad de La Frontera, Temuco, Chile.**
Development of a scaffold of PHB from *Paraburkholderia xenovorans* LB400 and gelatin for dermal tissue regeneration.

12:25 - 12:40 — **Dr. Olman Gómez-Espinoza, Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica, Costa Rica.**
Colobanthus quintesis under CO2 limitation: The response of oxalate oxidase and calcium oxalate crystals.

12:40 - 12:55 — **Dr. Kattia Núñez-Montero, Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica, Costa Rica.**
Sphingomonas alpina: A genomic study of psychrotolerant bacterial adaptations to the Antarctic continent.

Day 1 — Thursday, 25 November 2021 — Evening

Symposium II. Cellular and Molecular Biology of Reproduction
co-chairs: Dr. Ricardo Felmer Dörner & Dr. Maria Elena Arias Cea

15:00 - 15:30 — **Dr. Lleretny Rodríguez, Universidad de Concepción, sede Chillán, Chile.**
Embryo derived EVs: from embryo classification to embryo-maternal interaction.

15:30 - 15:50 — **PhD Student María José Contreras, Universidad de La Frontera, Temuco, Chile.**
Effect of cholestanol and cholesterol-loaded cyclodextrin on stallion sperm physiology and in vitro capacitation after cryopreservation.

15:50 - 16:10 — **Dr. Luis Águila Paredes, Universidad de La Frontera, Temuco, Chile.**
Haploid androgenetic development of bovine embryos: new insights of their developmental features.

16:10 - 16:30 — **Dr. Maria Gracia Gervasi, University of Massachusetts-Amherst, Amherst, Massachusetts, USA.**
Effects of sperm incubation conditions on in vitro early embryo development in the mouse.

16:30 - 16:50 — **Dr. Pamela Uribe Catalán, Universidad de La Frontera, Temuco, Chile.**
Analysis of autophagy in human sperm exposed to oxidative stress.

16:50 - 17:10 — **PhD Student Cecilia Valencia Robles, Universidad de La Frontera, Temuco, Chile.**
Effect of inhibition of protein synthesis and phosphorylation on bovine oocyte activation and embryonic development.

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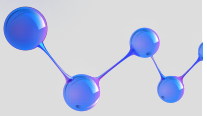

Workshop Programme

Day 2 — **Friday, 26 November 2021**
Morning

Symposium III. Cellular and Molecular Biology of Priority Diseases
Co-chairs: Dr. Cledir Rodrigues-Santos, Dr. Jorge Farias & Dr. Luis A. Salazar

09:00 - 09:30	**Dr. Manuel Varas Godoy, Universidad San Sebastián, Santiago, Chile.** Chemo-EVs and its contribution in tumor progression.
09:30 - 10:00	**Dr. Marcela Hermoso Ramello, Faculty of Medicine, ICBM, Universidad de Chile, Santiago, Chile.** IL-33/ST2 mechanisms controlling gut homeostasis that are deregulated in IBD.
10:00 - 10:30	**Dr. Gustavo Moraga-Cid, Faculty of Biological Sciences, University of Concepcion, Chile.** Glycine receptors as a therapeutic target in chronic pain: Deciphering the molecular determinants controlling, gating, conductance and sensitivity to allosteric modulators.
10:30 - 10:45	**PhD Student Luis Vergara Gómez, Universidad de La Frontera, Temuco, Chile.** Proteomic signature of gallbladder cancer exosomes: Deciphering its role in tumor progression and as reservoir of circulating biomarkers.
10:45 - 11:00	**PhD Student Martin Hödar Salazar, Universidad de La Frontera, Temuco, Chile.** Synthesis and Pharmacological Evaluation of Novel Multi-Target Nicotinic Ligands.
11:00 - 11:10	**Break**
11:10 - 11:25	**PhD Student Alberto Arencibia Núñez, Universidad de La Frontera, Temuco, Chile.** Transcriptomic landscape of Vascular Smooth Muscle Cell phenotypic switch.
11:25 - 11:40	**PhD Student Lisandra Herrera Belén, Universidad de La Frontera, Temuco, Chile.** Design and expression of chimeric L-asparaginase as a potential alternative for the treatment of Acute Lymphoblastic Leukemia.
11:40 - 11:55	**PhD Student Claudia Troncoso Muñoz, Universidad de La Frontera, Temuco, Chile.** Effect of *Helicobacter pylori* virulence on progranulin gene expression in gastric tissue of dyspeptic patients.
11:55 - 12:10	**Mr. Benjamin Durán-Vinet, Universidad de La Frontera, Temuco, Chile.** Towards next-generation colorectal cancer diagnosis: cancer exosomes-derived miRNA for CRISPR/Cas13-based detection.
12:10 - 12:40	**Dr. Constanza Martínez Cardozo, Pontificia Universidad Católica de Chile, Santiago, Chile.** Smoking habit does not affect the biological responses induced by leucocyte and platelet-rich fibrin.

Day 2 — **Friday, 26 November 2021**
Evening

Symposium IV. Applied Science: the challenge of linking academy and industry
Co-chairs: Dr. Luis A. Salazar Navarrete & Dr. Carmen Gloria Ili Gangas

14:30 - 15:00	**Mg. Franklin Valdebenito Godoy, DITT, Universidad de La Frontera, Temuco, Chile.** Trampolín Lab: A University program to foster science-based entrepreneurship.
15:00 - 15:20	**Dr. Claudio Lamilla Mardones, Universidad de La Frontera, Temuco, Chile.** Low environmental impact and low-cost detergent based on biosurfactants obtained from Antarctic bacteria.
15:20 - 15:40	**Dr. Daniela León Garrido, Universidad de La Frontera, Temuco, Chile.** Development of a cream for topical use with an improved formulation that ensures greater efficacy of photodynamic therapy in the treatment of non-melanoma skin cancer.
15:40 - 16:00	**Dr. Andrés Santos Ñanculef, Universitat Autònoma de Barcelona, Barcelona, Spain.** Early detection and monitoring of pathogenic microorganisms in the salmon industry.
16:00 - 16:10	**Break**

Day 2 — **Friday, 26 November 2021**
Evening

16:10 - 17:30	**Poster Presentation**
17:30 - 18:05	**Dr. Gabriela Repetto Lisboa, Centro de Genética y Genómica, Universidad del Desarrollo, Chile.** Advancing diagnosis for rare diseases through genomics.
18:05	**Dr. Luis Salazar Navarrete, Director of Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology.** Awards posters and Closing Ceremony.

Index

PREFACE

This book of abstracts contains the formal publications of the I International Workshop on Applied Cellular and Molecular Biology organized by the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology of the Universidad de La Frontera, held on the 25th and 26th November 2021 (Temuco, Chile). Given the complex and turbulence nature of the SARS-COV-2 pandemic at the global level, the Local Organising Committee of the Workshop together with the Direction of the Doctoral Program decided to organise it as a remote event.

The I International Workshop on Applied Cellular and Molecular Biology will cover the four major topics: 1) Biotechnology of Bioresources, 2) Cellular and Molecular Biology of Reproduction, 3) Cellular and Molecular Biology of Priority Diseases, and 4) Applied Science: the challenge of linking academy and industry. The first three scientific topics together with the knowledge development translated into industrial innovative products and services are important cornerstones for the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology.

Our Doctoral Program was established in 2005 by the Universidad de La Frontera (Res. Ex. 0310 27/01/2005) as a result of the evolution and disciplinary experience generated in the University for over a decade at that time. Nowadays, the Doctoral Program in Sciences, Major in Applied Cellular and Molecular Biology is one of the most recognised Doctoral Program in the field of Cellular and Molecular Biology in Chile. Its 103 alumni are currently contributing to the development of the country with basic and applied knowledge, in public and private organisations.

At a global level, cooperation involving students and academic staff have been developed over the last years, which has led to an increase in the number and quality of doctoral theses developed under both internship and double doctoral degree agreements with high quality institutions around the world. Also, foreign students from abroad have chosen Chile to develop their doctoral studies. In this context, this Doctoral Program has been selected by high quality foreign students to develop their doctoral theses, to develop an international doctoral internship or to develop their theses under a double doctoral degree. All of these actions have had a positive influence on the increase in scientific productivity and, consequently, on the quality impact of generated publications and knowledge.

The I International Workshop on Applied Cellular and Molecular Biology is indeed a good opportunity to congregate altogether our students based in Chile, our students from abroad that have already developed or will develop their internship in our Doctoral Program, our academic staff and also our academic colleagues from national and foreign institutions.

We are delighted at the participation of students, researchers and professors from 14 countries (Argentina, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Italy, Panama, Mexico, Peru, United States, Venezuela, Spain and Chile) and the more than 200 attendants resulting in excellent 20 keynotes, 10 oral communications and more than 30 posters. These numbers confirm the I International Workshop on Applied Cellular and Molecular Biology will be a very significant international conference.

Thanks are due to the many individuals who have been involved at all levels of the planning, coordinating, reviewing, organising and convening of the symposia. The virtual meeting facilities and, last but not the least, the Agencia de Comunicaciones Xplain (Chile) are also acknowledged.

Finally, we wish you a very productive meeting and hope to see you personally very soon in Temuco.

Organizing Committee

Opening conference

Solving real-life problems through Genomics and Bioinformatics.

Maracaja-Coutinho, Vinicius

Departamento de Bioquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile; Advanced Center for Chronic Diseases – ACCDiS, Santiago, Chile.

Bioinformatics is a transversal discipline that is based on the integration of information technologies, mathematics and statistics for the management, understanding and analysis of biological data. The areas related to bioinformatics have been developing with increasing interest and impact since the 70s and are consolidated in the 2000s with the publication of the first draft of the human genome. From there, the contributions of bioinformatics have permeated various sectors of industry, including agriculture, mining, aquaculture and medicine. In this opportunity, we will present different contributions that genomics and bioinformatics has given us to solve real-life problems, related to climate change, economy, agroindustry, aquaculture and human health.

Symposium I: Biotechnology of Bioresources

The Role of Mitochondrial Metabolism in Plant Stress Responses.

Nunes-Nesi, Adriano

Departamento de Biologia Vegetal, Universidade Federal de Viçosa
36570-900 Viçosa, MG, Brasil.

In the future, CO2 concentrations, together with the temperature, are expected to be considerably higher than today. Drought periods are likely to be more frequent and severe, while flooding also increases, leading to soil degradation and increased incidence of climatic extreme events. Thus, understanding the impact of ongoing climate change on crops physiology and performance is essential for predicting future yields and ensuring food security. It has been demonstrated in plants that mitochondrial metabolism is integrated with other essential processes such as photosynthesis and photorespiration. These studies suggest that mitochondrial proteins participate in the regulation of stomatal opening and development, nitrogen metabolism, as well as changes in leaf redox potential. During my presentation, I will discuss the mechanisms by which mitochondria modulate growth and photosynthesis in plants.

Financial support: *This work was supported by funding from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; grant number CRA-RED00053-16).*

Small temperature changes act as drivers for metabolic reprogramming of *C. quitensis* plant and associated microbes in the Antarctic scenario.

Bertini, Laura

Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy.

Incessant advancements in "omics" technologies have opened up new perspectives to achieve a comprehensive understanding of biological processes related to stress responses in plants. In this presentation, the application of "omics" disciplines to discerning the effect of global warming on the survival of *C. quitensis* Antarctic ecotype will be showed. Exploiting a simulated global warming experiment set up in King George Island, South Shetland archipelago, we combined proteomic and metabolomic analyses to compare the metabolic reprogramming of *C. quitensis* plants grown in open area (OA samples) versus plants grown inside Open Top Chambers (OTCs samples). These small greenhouses open on the top determine an increase of about 3 °C during midday, allowing to mimic the effect of global warming. Interestingly, we found that in OTCs samples photorespiration could play an important role in reducing oxidative stress and ROS-mediated photodamage improving protection against photoinhibition. A higher protection against oxidative stress in plants grown under warmer conditions was also highlighted by the metabolomic analysis, which revealed an increased production of antioxidant-related metabolites in OTCs samples. In addition, activity assays of ROS scavenger enzymes corroborated the evidence that OTCs samples deploy stronger antioxidant defenses. Moreover, we applied metatranscriptomics and culturomics approaches to characterize the fungal community associated to *C. quitensis* leaves at both functional and species level, respectively. Newsworthy, we found higher fungal metabolic and transcriptional activities in plants grown under warmer conditions and many of them were found to be involved in the biosynthesis of compounds functional to plant growth. Finally, we isolated culturable endophytic fungi that are currently under study in order to evaluate their possible biotechnological exploitation.

Implications of adhesin discovery in the emergent yeast pathogen *Trichosporon asahii*.

Barbosa-Padovan, Ana Carolina

Department of Microbiology and Immunology, Federal University of Alfenas, Alfenas, Brazil.

Trichosporon asahii is an emergent opportunistic yeast-like pathogen from the *Basidiomycota phylum.* This species is able to cause both mild, superficial infections in the skin and hair follicles (i.e. *piedra branca*) and also severe deep seated infections, known as invasive trichosporonosis in immunodeficient patients. That type of infection presents extremely high mortality rates that reach up to 90%, also attributed to the emergence of antifungal resistance. The chain of events that leads up to a successful infection must include adhesion of the fungi to its host as the first step of colonization. Adhesins are fungal glycosylated proteins responsible for such interaction and therefore must be studied in order to further understand the pathogenesis. They are secreted to the out layers of the cell and can be anchored in the cell membrane or cell wall. Their expression during fungal cell-to-cell interaction (flocculation), mediating the fungal adherence to human cells, and during biofilm formation designate them as important virulence factors. Intriguingly, any adhesins are known in the emergent pathogen *T. asahii.* The talk will focus on the discovery and characterization of putative adhesins that are differentially expressed in yeasts, hyphae and biofilms of *T. asahii* depending on the clinical isolation site and explore their ligands within the human host.

Molecular diagnosis of sporotrichosis: What methodologies are applied for the identification of species in our Research Group?.

Oliveira, Manoel

Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

Sporotrichosis is a subcutaneous mycosis with worldwide distribution and until 2006 only *Sporothrix schencki,* was considered the causative agent of the disease. From there, was described the *Sporothrix* complex and new species. *Sporothrix* sp. are dimorphic fungi, filamentous in environmental or in culture at 25°C and yeast form in parasitism or culture at 37°C. Several animals, as domestic mammals, are susceptible to *Sporothrix* and may be infected and cats are the most affected. Actually, eight species were described in *Sporothrix* complex. In Brazil *S.brasiliensis* is the prevalent etiological agent, infecting humans, cats and dogs. Feline cases are also caused by *S.schenckii* and *S.pallida* and canine by *S.schenckii* and *S. luriei.* People are also infected by *S. schenckii, S. globosa, S. mexicana* and *S. chiliensis.* Human and animal diagnosis are based on patient history, clinical signs, epidemiological data and laboratorial exams. Cytology and histopathology are very useful routine tools for the preliminary diagnosis, however, the definitive is obtained by culture and isolation of *Sporothrix* sp.. Immunohistochemistry, serology and PCR are other options for the diagnosis of sporotrichosis, usual in research. The aim of this presentation is highlighting the fast and cheap methodologies developed by my research group for identification of species of *Sporothrix* complex. The fast PCR Fingerprinting using a universal primer for T3B is a methodology that allows the identification of species with profile defined and possible to use in the routine. MALDI-TOF MS is other fast, cheap, simple, reliable and accurate tool, that reduces time of identification, whose protocol was standardized in our laboratory in support of other groups in diagnosis of sporotrichosis. And finish the presentation I will answer the question: There are molecular tools for direct diagnosis of sporotrichosis using clinical samples, after description of complex Sporothrix by polyphasic taxonomy?

Natural and semisynthetic bioactive drimane sesquiterpenoids isolated from *Drimys winteri*.

Burgos, Viviana [1]*; Durán, Paola* [1]*;* ***Paz, Cristian*** [1]

[1] *Universidad de La Frontera, Temuco, Chile.*

Natural compounds are a valuable source of medicines and have been traditionally used by First Nations people for the treatment of diverse diseases. For example, the *Mapuche* people in Chile consider to the tree *Drimys winteri* as sacred, and its leaves and barks are used by the *machi* in the treatment of skin injuries and different affections. *Drimys winteri* is rich in bioactive drimane sesquiterpenoids, which are formed by a central trans-decalin bicycle of 10 carbons. Positions 11 and 12 of the drimane skeleton are usually functionalized with alcohols or aldehydes, with the possibility to form an additional third ring of lactone. The biological activity of each compound is different, for example drimane sesquiterpene lactones as cinnamolide or drimenin have showed inhibition activity of nicotinic acetylcholine receptors (nAChRs), involved in depression and drug addiction. On another hand, polygodial, which is a 1,4-dialdehyde has showed potent antifungal activity, for instance, against the human parasitic yeast *Candida albicans,* polygodial displays a MIC of 3 µg/mL, as well as against other fungal strains as B. cinerea and Ggt. In addition to trypanocide and insecticidal properties, but despite these activities, polygodial has a pungent flavor due to activation of TRP channels, together with irritant properties in eyes and soft skin, that make its uses unviable. Moreover, we determinate recently that polygodial inhibit the sodium channel Nav1.7 and Nav1.8, responsible for the pain sensation, which could be attributed to the reactivity of dialdehydes to amines, forming a pyrrole moiety that causes a covalent interaction with key proteins. Currently, we are working in the development of new nAChRs ligands for depression therapy, as well as the synthesis of new antifungal compounds with less side effects than polygodial, which could be used as adjuvant in the treatment of Candidiasis.

Development of a scaffold of PHB from *Paraburkholderia xenovorans* LB400 and gelatin for dermal tissue regeneration.

Sanhueza, Claudia [1]*; Hermosilla, Jeyson* [2]*; Acevedo, Francisca* [3,4]

[1]*Centro de medicina traslacional, Universidad de La Frontera, Temuco, Chile.*
[2]*Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile.*
[3]*Departamento de Ciencias Básicas, Universidad de La Frontera, Temuco, Chile.*
[4]*Núcleo Científico Tecnológico en Biorecursos, Universidad de La Frontera, Temuco, Chile.*

The extracellular matrix of the skin should provide mechanical support, regulates cellular activities and may facilitate the transportation of the nutrient and metabolic wastes needed for cells to survive and function effectively. Scaffolds should resemble not only the composition but also the topography and properties of the ECM. Polyhydroxybutyrate (PHB) is a hydrophobic polyester produced by a wide variety of bacterial using different carbon sources. *Paraburkholderia xenovorans* LB400 has been described as a PHB producer using glucose, xylose and mannitol as sole carbon sources. PHB is a biodegradable and biocompatible polymer, which makes it suitable for biomedical applications; however, the biodegradation rate of PHB is low. Different studies in tissue engineering area have been developed combining PHB with gelatin (Ge), which is a hydrolyzed collagen highly used in tissue engineering that provides a better environment for cell attachment, growth, and proliferation. Thus, the aim of this work was to evaluate the physicochemical and biological properties of an electrospun skin scaffold developed with the combination of nano and microfibers of Ge and PHB from *P. xenovorans* LB400 using xylose as the carbon source (PHB_x). Physico-mechanical properties, swelling, hydrophilicity of electrospun scaffolds made of PHB_x, commercial PHB microfibers (PHB_c), Ge-nanofibers and their combination (Ge-PHB_x and Ge-PHB_c) were evaluated. The biological properties of the scaffolds were evaluated *in vitro,* in fibroblast Balb 3T3 and *in vivo*, in wounds caused by secondary intention in diabetic rats. It was possible to combine PHB_x-microfibers and Ge-nanofibers simultaneously by electrospinning. Relevantly, PHB_x-microfibers exhibited mechanical characteristics similar to those of human skin, despite their low morphology index. *In vitro* tests showed that the Ge-PHBx scaffold is highly compatible with the tested cell line, with values over 100% at day 7. At day 3 and day 7, significant differences were observed between Ge-PHB_x and Ge-PHB_c. Ge-PHB_x showed a significantly higher fibroblast viability than Ge-PHB_c. In the *in vivo* assay, no significant differences were observed. However, at day 7 two main subsets were observed, where it can be emphasized that the Ge-PHB_x scaffold achieved a clear improvement compared with the others scaffolds evaluated. The results of this study indicate that the use of fibers manufactured by electrospinning with PHB_x from *P. xenovorans* LB400 produced a scaffold with improved biological and mechanical properties as compared with those created from commercial-PHB.

Colobanthus quintesis under CO2 limitation: The response of oxalate oxidase and calcium oxalate crystals.

Gomez-Espinoza, Olman [1]*; Bravo, Leon A.* [2]

[1]*Centro de Investigación en Biotecnología, Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica.*
[2]*Laboratorio de Fisiología y Biología Molecular Vegetal, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias, Universidad de La Frontera, Temuco, Chile.*

The leaves of the Antarctic plant *Colobanthus quitensis* exhibit a reduced CO2 diffusion, resulting in photosynthetic limitations. Nonetheless, this plant optimizes its carbon assimilation to survive the harsh Antarctic environment; but the mechanism used for *C. quitensis* to countervail the CO2 diffusion is unclear. We tested whether C. quitensis possess oxalate oxidase enzymes (OxO), whose activity could be associated with the decomposition of calcium oxalate crystals to obtain CO2, maintaining a basal level of photosynthesis, as it has been recently evidenced in other plants and proposed as an "Alarm photosynthesis". Putative OxO enzymes were identified from the transcriptome by in silico analysis using phylogeny and docking tools. In addition, *C. quitensis* plants were placed in airtight chambers injected either with ambient air (~400ppm CO2) or soda lime filtered air (~10ppm CO2) for 10 hours. Crystal's areas in leaves were monitored using polarized-light microscopy and digital image analysis; measurements of electron transport rate (ETR) and OxO activity were also performed for both treatments. A significant reduction in leaf crystals area was observed in the CO2-limited condition at the end of the experiment. Crystal decomposition was accompanied by increased OxO activity and a slight decrease in ETR. Our results suggested that a CO2 limiting condition is directly related with CaOx crystals decomposition. Consequently, to compensate the reduced CO2 diffusion of leaves developed under its natural habitat, the CaOx crystal decomposition might play a role as a complementary endogenous mechanism facilitating the CO2 supply in the Antarctic plant *C. quitensis.*

Sphingomonas alpina: A genomic study of psychrotolerant bacterial adaptations to the Antarctic continent.

Núñez-Montero, Kattia [1]*; Barrientos, Leticia* [2]

[1]*Centro de Investigación en Biotecnología, Escuela de Biología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica.*
[2]*Laboratory of Molecular Applied Biology, Center of Excellence in Translational Medicine, Universidad de La Frontera, Avenida Alemania 0458, Temuco 4810296, Chile*

Strains from the family Sphingomonadaceae have a great potential for biotechnological applications, including bioremediation, degradation of emergent pollutants and production of biopolymers called sphinganes-used as plant growth promoters-. The genus *Sphingomonas* has been characterized a soil improver, showing a environmental role in the recycling of complex molecules. Due to its capacity to accumulate heavy metals, it improves plant resistance in environments with high Zinc, Cadmium and Copper contents. In this work we aimed to characterize the differential genomic elements of the Antarctic bacterium *Sphingomonas alpina* So64.6. The DNA of the Antarctic strain was sequenced with Illumina and ONT technologies to obtain its complete genome, which was studied to determine the differential characteristics of the Antarctic strain when compared to reference genomes of the genus. For this purpose, phylogenetic constructions based on the 16SrRNA gene, proteome and core genome, as well as a pangenome analysis were performed. Phylogenetic results suggest that this strain corresponds to *S. alpina* species, however, they showed important variations in the average nucleotide identity due to differential accessory genes between both strains. The Antarctic strain So64.6b showed unique transposase proteins, insertion elements and proteins related to contaminant degradation including benzoate, gallate, lignin, xylan and tetrahydrofolate, which were not found in the reference alpine strain. The results also highlighted the dynamism of this group, as many of these genes were found in genomic islands. This strain also showed a high diversity of biosynthetic clusters potentially associated with the production of novel antibiotic molecules and a high capacity for metal resistance, characteristics that seem to be unique to the Antarctic strain and absent even in other strains of the same species. Our work highlights the influence that the selective pressure of the Antarctic environment may have exerted on the evolution and genetic conformation of the Antarctic strain under study.

Acknowledgment: Beca Doctoral CONICYT– PFCHA/Doctorado Nacional/2017–21170263, INACH DG_19–01, NEXER NXR17-0003.

Symposium II: Cellular and Molecular Biology of Reproduction

Embryo derived EVs: from embryo classification to embryo-maternal interaction.

Rodriguez-Alvarez, Lleretny

Laboratorio de Biotecnología Animal, Facultad de Ciencias Veterinarias, Universidad de Concepción, Concepción, Chile.

Extracellular vesicles (EVs) are currently considered a mechanism of cell communication. EVs are nanoparticles surrounded by a lipid bilayer and they carry bioactive molecules such as proteins, lipids, DNA, mRNA, and miRNA. These are secreted from different cell types and their characteristics (size, concentration and cargo) vary according to the physiological or pathological state of the cell. EVs can be identified in vivo in different biological fluids, as well as in medium from cells in culture. EVs are also identified in culture media from in vitro-produced embryos of different species including porcine, bovine, mouse and human. It seems that embryo-derived EVs participate in the early embryo-maternal interaction to prepare the maternal side for pregnancy recognition and implantation. EVs secreted by bovine embryos are internalized by endometrial cells, inducing the expression of interferon tau-stimulated genes. On the other hand, the cargo and morphological parameters of EVs (size mean and concentration) vary during preimplantation development and as consequence of the technology (in vivo or in vitro production) used for embryo production and embryo competence. Based on that, embryo-derived EVs have been suggested as non-invasive markers for embryo selection. For instance, the average concentration of EVs secreted during compaction by individual bovine embryos is significantly lower than the concentration of EVs secreted during blastulation and hatching. Furthermore, competent embryos secrete during compaction lower amount and smaller EVs while during blastulation, competent embryos secrete less but bigger EVs. Additionally, 8 miRNAs are differentially expressed between EVs secreted by competent and non-competent embryos during compaction while 8 miRNAs are only detected in EVs secreted by competent embryos during blastulation. These miRNAs might be appropriate candidate markers for selection of best quality embryos. Moreover, EVs contain embryonic DNA that can be used for preimplantation genetic diagnosis of in vitro produced embryos.

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Effect of cholestanol and cholesterol-loaded-cyclodextrin on physiology and *in vitro* capacitation of stallion sperm after cryopreservation.

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Cryopreservation of stallion semen is inefficient and does not show the positive results described in other species. This is because of the greater susceptibility of stallion sperm to the freezing damage that generates oxidative stress and plasma membrane injury, resulting in DNA fragmentation and cell death. These data suggest the need of finding new strategies of sperm cryopreservation that can improve the efficiency of this technique in stallion by preventing the membrane damage and cell death. The present study aimed to evaluate the effect of adding membrane stabilizers to the freezing medium and assess the quality and in vitro capacitation of stallion sperm after thawing. Samples from three stallions frozen with membrane stabilizers (cholesterol-loaded cyclodextrin and cholestanol-loaded cyclodextrin) were evaluated in two experiments: i) sperm quality analysis after thawed, and ii) sperm quality and functional analysis after 4 h of post-thawed incubation in capacitation conditions. Plasma membrane integrity, mitochondrial membrane potential, membrane lipid disorder, intracellular $^{Ca2+}$, tyrosine phosphorylation, acrosome reaction, DNA damage, sperm motility, and binding to the zona pellucida were assessed. The results showed that cholesterol loaded cyclodextrin was the stabilizer that best controlled the membrane disruption and post-thaw cell damage. In addition, this stabilizer allowed to obtain in vitro capacitated sperm showing higher plasma membrane integrity, mitochondrial membrane potential, sperm motility, number of spermatozoa bound to the zona pellucida and better response to in vitro capacitation conditions.

Haploid androgenetic development of bovine embryos: new insights of their developmental features.

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Uniparental haploid embryos are efficient models to study genome imprinting and to understand the contribution of the parental genome to early embryonic development. However, bovine haploid androgenetic embryos (hAE) show poor developmental potential compared to haploid parthenogenetic (hPE) and/or biparental embryos. Thus, our objective was to characterize the developmental constraints of bovine haploid androgenetic embryos by analyzing their developmental features according to expression levels of paternal-specific long noncoding RNAs, some maternally expressed imprinted genes, and pluripotency-related genes, as well as the cell number and their allocation at the blastocyst stage. We produced bovine hAE by intracytoplasmic sperm injection (ICSI) using X-sorted sperm, which were compared to hPE and biparental embryos. The results showed that XIST transcripts were significantly more abundant in hAE than in all other groups at the morula-stage. Moreover, transcripts of the KNCQ1OT1 lncRNA and the CDKN1C, PHLDA2, and TSSC4 imprinted genes were upregulated in hAE, suggesting a disturbed epigenetic regulation. In addition, the hAE had altered expression of NANOG, CDX2, KFL4, and SOX2 at the morula stage ($p<0.05$), but only NANOG remained altered (overexpressed) at the blastocyst stage ($p<0.05$). Finally, bovine hAE showed a lower number of cells (75 for hAE vs 123 and 134 for hPE and ICSI, respectively ($p<0.05$) and flawed inner cell mass formation (6% for hAE vs 27% and 29% for hPE and ICSI, respectively). Our data indicate that the failure of haploid androgenetic embryos to develop to the blastocyst stage is associated with abnormal expression of key lncRNAs involved in XCI and genomic imprinting at the KCNQ1 locus, dysregulated expression of pluripotency-related genes and deficient cell differentiation during blastocyst formation.

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Effects of sperm incubation conditions on in vitro early embryo development in the mouse.

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Sperm capacitation consists of all the changes that sperm undergo while in the female reproductive tract that render them able to fertilize an oocyte. The process of in vitro fertilization (IVF) relies on the ability to capacitate sperm in vitro. Assisted Reproductive Technologies, like IVF, are valuable techniques used to treat infertility in both human and animals. We have recently developed the Sperm Energy Recovery after starvation (SER) treatment prior to IVF, that has proven to increase the rates of fertilization and in vitro blastocyst development in the mouse. We hypothesized that SER treatment prior to IVF affects the developmental potential of the early embryo by an epigenetic mechanism. We studied epigenetic differences of the pronuclei by comparing early stages of development of embryos obtained by IVF with SER-treated and control sperm. We focused on the histone modification H3K4me3 that is associated with the activation of gene expression. We found that all the SER-derived embryos displayed H3K4me3 in the male pronuclei at 12 hours post insemination while only a percentage of control derived embryos displayed H3K4me3. We found that the population of control-derived embryos were more heterogenous as opposed to the homogenous SER-derived population. The heterogeneity in the control embryo population might be related to the lower developmental potential to blastocyst stage. Our results indicate that the sperm SER treatment prior to IVF alters epigenetic markers in the male pronuclei and this could be related to the improved developmental potential of the embryos.

Analysis of autophagy in human sperm exposed to oxidative stress.

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Autophagy is a regulated pathway of lysosomal degradation which helps eukaryotic cells to maintain or restore homeostasis, being a cellular stress response mechanism. Oxidative stress (OS) is a main cause of impaired sperm function and linked to male infertility; however, the ability of OS to activate autophagy in human spermatozoa is unknown. The main objective here was to evaluate the autophagic response in OS-exposed human spermatozoa. For this study semen samples from normozoospermic donors were used and motile sperm cells were selected by the swim up technique. Human spermatozoa were exposed separately to ionomycin and hydrogen peroxide in order to induce OS. An untreated control group was included. First, the generation of OS under our experimental conditions was demonstrated by analyzing viability, reactive oxygen species (ROS) production, mitochondrial membrane potential ($\Delta\Psi m$) and motility. Then, proteins involved in autophagy including LC3-I, LC3II, ATG5 and ATG16 were analyzed in spermatozoa exposed to OS and compared to the untreated control. Then, spermatozoa under OS were exposed to chloroquine in order to block autophagy. An untreated control and a control incubated only with the OS inducer were included in each experimental setting. Viability, $\Delta\Psi m$, phosphatidylserine externalization and caspase activation were analyzed. The results showed that the exposure to ionomycin and hydrogen peroxide promotes OS resulting in increased ROS production and decreased viability, $\Delta\Psi m$ and motility. These alterations were accompanied by a decrease in LC3-I, indicating that autophagy was activated upon OS exposure. Ionomycin also caused an increase in LC3-II, ATG5 and ATG16 expression. Autophagy blocking of sperm exposed to OS caused deterioration in sperm quality, metabolic parameters as well as an increase in cell death markers. These results suggest a crucial role of autophagy as a stress response by male gametes, and which contributes to maintaining the functionality and lifespan of ejaculated sperm cells.

Effect of inhibition of protein synthesis and phosphorylation on bovine oocyte activation and embryonic development.

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In equine and porcine to improve efficiency of oocyte activation, combined use of inhibitors of protein-synthesis and phosphorylation has been described, however, there are not enough studies in bovines. In present study we compared the effect of synthesis-inhibitors (ANY and CHX) and protein-phosphorylation (DMAP) and its combination (ANY; ANY+CHX; ANY+DMAP; ANY+CHX+DMAP; CHX+DMAP) on parthenogenetic activation of bovine oocytes, assessing the embryonic development up to blastocyst stage under stereo microscope and expression and status of maturation-promoting-factor (MPF) components (cyclin-B1, CDK1, p-CDK1Thr161 and p-CDK1Thr14-Tyr15) and ERK1/2 pathway by immunodetection at 4 hours-post-activation. Results not showed differences between treatments in cleavage rate (91.6-98.6%) and blastocyst rate (37.4-50.9%). Moreover, the treatments did not modify the level expression of cyclin-B1 and total CDK1, nor p-CDK1Thr161 and p-CDK1Thr14-Tyr15. However, decrease expression of ERK1/2 protein was observed in oocytes treated with ANY and ANY+CHX, compared to ANY+DMAP, ANY+CHX+DMAP and CHX+DMAP treatments ($p<0.05$). Besides, decrease p-ERK1/2 was observed in ANY+DMAP, ANY+CHX+DMAP and CHX+DMAP, compared to ANY and ANY+CHX ($p<0.05$).
The results indicate that the activation of bovine oocytes with treatments used in the present experimental design does not occur by decrease of cyclin-B1. Also, since the levels of activating (p-CDK1Thr161) and inhibitory (p-CDK1Thr14-Tyr15) phosphorylation of MPF were observed in balance, we infer that MPF is present in an inactive form (preMPF). Moreover, protein-synthesis-inhibitors alone (ANY) or in combination (ANY+CHX), generated decrease of ERK1/2 on a similar level. While protein phosphorylation inhibitor (DMAP) combined with ANY, CHX or both, decreased phosphorylation of MAPK, compared to treatments with protein synthesis inhibitors. In conclusion, treatments used favors the embryonic development and promote the activation of bovine oocytes in ERK1/2-dependent manner. Futures analyses of gene expression are necessary to assess the treatments effect on embryonic quality.

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Symposium III: Cellular and Molecular Biology of Priority Diseases

Chemo-EVs and its contribution in tumor progression.

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Different studies support the idea that extracellular vesicles (EVs) could modify other cells to induce chemoresistance or metastasis, and in this context different stimulus such as chemotherapy promote the secretion of EVs (Chemo-EVs) with pro-tumorigenic activity. Results of our lab using chemoresistance ovarian cancer cells (A2780cis or tumor spheres) show that cisplatin induce release of Chemo-EVs from these cellular models with the capability to transfer chemoresistance to sensitive cancer cells, and also promote a pro-tumoral phenotype of mesenchymal stem cells (MSCs) which are important in the tumor progression. We investigated the protein contents of the Chemo-EVs and we found proteins related to stabilization of p53, regulation of RUNX2 and PTEN, metabolism of RNA, and DNA double-strand break repair which could be responsible for the pro-tumorigenic effects. In conclusion, EVs released by chemoresistance ovarian cancer cells are modified in response to cisplatin and acquire pro-tumorigenic activity modifying its contents.

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IL-33/ST2 mechanisms controlling gut homeostasis that are deregulated in IBD.

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Chronic inflammation and loss of intestinal homeostasis are implicated in inflammatory bowel diseases (IBD), including "deregulation" of the well-known alarmin interleukin-33 axis (IL33/ST2). Interestingly, ST2 deficient mice are more susceptible to chemical-induced colitis, highlighting its central role in intestinal inflammation. Accordingly, understanding how IL-33/ST2 axis contributes to intestinal mucosal inflammation, will hopefully allow its use as a potential biomarker or therapy target in IBD.ST2 gene (IL1RL1) of the Toll-like /interleukin-1 receptor superfamily (TLR/IL-1R), mainly encodes two isoforms, ST2L and soluble ST2 (sST2). IL-33 binds to membrane anchored ST2L promoting tolerogenic and wound healing response genes. Additionally, sST2 is a decoy receptor and inflammatory mediator that we have identified in intestinal mucosa and plasma of active ulcerative colitis (UC) patients, suggesting ST2 as a potential biomarker. Based on sST2 production by inflamed intestinal mucosa, cells involved in IBD development and progression might provide altered ST2/IL-33 component expression, contributing to pathogenic response perpetuation, and thus reducing anti-inflammatory IL-33-driven ST2+ Treg cell function. Recently, we found that sST2-circulating levels correlate with therapy response, being higher in UC patients under corticosteroids. Moreover, due to IBD patient refractoriness to conventional therapy, we have developed a specific antibody against ST2, reversing inflammation in in vitro models. Thus, modulation of IL-33/ST2 axis to induce and/or maintain remission in IBD is a promising therapeutic target, with sST2 being an appealing candidate.

Glycine receptors as a therapeutic target in chronic pain: Deciphering the molecular determinants controlling, gating, conductance and sensitivity to allosteric modulators.

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Glycine receptors (GlyRs) are anion-permeable ligand-gated ion channels of the Cys-loop superfamily. In the mammalian CNS, the enhancement of the chloride conductance through the activation of GlyRs results in a transient hyperpolarization of the membrane potential, which is critical to control the neuronal excitability. The relevance of glycinergic inhibition is underlined by the presence of malfunctional GlyRs in many pathophysiological states, including chronic pain. Previous studies have shown that the dysfunction of GlyRs containing the alpha 3 subunit is a pivotal mechanism of pain hypersensitivity. This pathway involves the activation of prostaglandin receptors and the subsequent PKA-dependent phosphorylation of alpha 3 GlyRs within the intracellular domain (ICD), which decrease the GlyR-associated currents and in turn enhance the neuronal excitability. Despite the importance of this pain sensitization pathway associated with the dysfunctional alpha 3 GlyRs, our current understanding of the molecular events involved is very limited. Here we report that PKA-mediated phosphorylation of alpha 3 GlyR decreases the ion channel conductance. We show in addition that the substitution of the PKA-targeted serine with a negatively charged residue within the ICD of alpha 3 GlyRs and of chimeric GLIC-GlyR receptors was necessary and sufficient to generate receptors with impaired conductance. Furthermore, we show that a recently characterized GlyR modulators showing in vivo analgesic activity normalized the impaired conductance of phospho-mimetic alpha 3 GlyRs. Our findings thus propose a molecular framework for a pain sensitization mechanism involving neuronal dis-inhibition and suggest that the allosteric modulation of alpha 3 GlyR alleviates chronic pain at least in part through the restoration of phosphorylated ion channels with impaired chloride conductance.

Proteomic signature of gallbladder cancer exosomes: Deciphering its role in tumor progression and as reservoir of circulating biomarkers.

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Gallbladder cancer (GBC) is an aggressive neoplasm diagnosed in advanced stages and characterized by a poor prognosis and insufficient number of therapeutic target and biomarkers for early detection and tumor progression. Exosomes are extracellular vesicles (EVs) released by neoplastic and non-neoplastic cells into different body fluids with a selective molecular content that is used as a cellular communication mechanism. In tumor cells, EVs carry molecules such as nucleic acids and oncogenic proteins to promote the malignant cellular transformation. To characterize the proteomic profile of EVs derived from GBC cells as well as to determining their relevance in the tumor progression and as a source of potential biomarkers and therapeutic target. EVs released by eight GBC cell lines were isolated from conditioned medium using ultracentrifugation and, subsequently, their morphology and abundance were characterized by Nanotracking Particle Analysis and Transmission Electron Microscopy. EVs proteome was obtained using mass spectrometry and ontologically described by bioinformatics tools. EVs showed variability in size, abundance and protein composition among the eight GBC cell lines studied. Analyzing the overexpressed proteins, we found membrane proteins and extra and intracellular proteins involved functionally in transport, cellular communication and signal transduction. Recognized exosomal markers (GTPases, Tetraspanins, FLOT1, FLOT2, TSG101) and potential therapy targets (MIF, HSP90, S100A2) were identified. Besides, we observed shared proteins between the vesicular proteome and total proteome of GBC cell lines, which suggest that composition of EVs is representative of their progenitor tumor cells. EVs proteome provides a source of protein that could be validated as biomarkers and therapy targets to improve clinical outcomes in patients. In addition, using liquid biopsy, EVs could be obtained from body fluids as a non-invasive strategy for early diagnosis and clinical following of GBC.

Synthesis and Pharmacological Evaluation of Novel Multi-Target Nicotinic Ligands.

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The monoaminergic and cholinergic neurotransmitter systems exhibit, in the central nervous system (CNS), a wide range of functional interactions and mutual regulations. Furthermore, acetylcholine (ACh) actions mediated by nicotinic receptors (nAChRs), as well as monoamines such as serotonin (5-HT) and dopamine (DA), are involved in the modulation of several brain functions, including (but not limited to) cognition, voluntary movement, motivation and reward, mood, attention and learning, as well as in the physiopathology of a variety of diseases. In addition, most of the drugs currently used for the treatment of neurological and neuropsychiatric disorders such as Parkinson's and Alzheimer's diseases, depression, drug addiction and schizophrenia, have mechanisms of action associated to the simultaneous regulation of these neurotransmitters. Here, we design and synthesize cycloalkylamines, piperidines and N-methyl-pyrrolidine ester derivatives. Binding experiments were assessed for a4b2 nAChR on brain synaptosomes and hSERT and hDAT from specific cell lines. Our results indicate that, some compounds are able to displace radioligands from nicotinic receptor and MAT, showing a promiscuous behavior, presenting affinities were in the micromolar order. In addition, docking experiments were performed in order to rationalize the binding mode and the similar interaction between α4β2 nAChR, SERT and DAT with our compounds.

Transcriptomic landscape of Vascular Smooth Muscle Cell phenotypic switch.

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Coronary in-stent restenosis is a late complication of angioplasty. It is a multifactorial process that involve vascular smooth muscle cells (VSMCs), endothelial cells, inflammatory and genetic factors. The transcriptomic landscape of VSMCs phenotypic switch process under stimuli resembling stent injury was assessed in this study. Co-cultured contractile VSMCs and endothelial cells were exposed to bare metal stent and platelet derived growing factor (PDGF-BB) 20ng/ml. Migratory (wound healing assay), proliferative capacity and cell cycle analysis of VSMCs were performed. RNAseq analysis of contractile vs. proliferative VSMCs was performed. Gene differential expression (DE), identification of new long non-coding RNAs (lncRNAs) candidates and gene ontology (GO) enrichment were analyzed. VSMCs exposed to "stent injury" conditions showed morphologic changes, with proliferative and migratory capacities, progressing from G0-G1 cell cycle phase to S and G2-M. RNAseq analysis showed DE of 3517 mRNAs and 2911 ncRNAs. Using more stringent criteria we identified 1099, 509 and 64 differentially expressed mRNAs, lncRNAs and microRNAs respectively. lncRNAs were mostly upregulated under experimental conditions while mRNAs were downregulated. Among the top twenty disregulated LncRNAs there were eight not previously annotated. GO analysis of DE mRNAs showed significant enrichment for collagen and extracellular matrix organization, chemokine-mediated signaling pathway and regulation of smooth muscle cell proliferation. GO analysis for mRNA-lncRNA target genes showed enrichment for terms related to wound healing, collagen biosynthetic process and endothelial cell proliferation in angiogenesis. VSMCs under our experimental conditions developed a proliferative phenotype. The transcriptomic landscape of this cells shows a particular hallmark with upregulation of lncRNAs, some of them identified as potentially new transcripts. The genetic signature of the phenotypic switch of VSMCs could serve as a basis for a pipeline of genetic biomarkers discovery, allowing an early diagnosis of post-angioplasty coronary stent restenosis.

Design and expression of chimeric L-asparaginase as a potential alternative for the treatment of Acute Lymphoblastic Leukemia.

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Acute Lymphoblastic Leukemia (ALL) represents the most common cancer in the childhood population worldwide, which is characterized by an overproduction of undifferentiated lymphoblasts in the bone marrow. Against this disease, the treatment of choice is the enzyme L-asparaginase (ASNase), isolated from *Escherichia coli* and *Dickeya chrysanthemi.* The action of this enzyme consists of the hydrolysis of circulating L-asparagine in plasma, leading to the formation of aspartic acid and ammonia. Due to leukemic cells' inability to synthesize enough L-asparagine for survival, they take L-asparagine from blood plasma, so the action of ASNase causes apoptosis of these cells by starvation. In addition, the ASNase formulations of *E. coli* and *D. chrysantemi* present several immunological adverse effects. For these reasons, various chemical and genetic engineering strategies have been adopted to minimize these side effects. In this work, we present the expression of chimeric asparaginase. This enzyme was generated by substituting the antigenic epitopes of the *E. coli* ASNase for structurally equivalent regions of a human enzyme. Through bioinformatics tools, the chimeric enzyme was designed *in silico* and later expressed with activity in the periplasmic fraction in host cells of *E. coli.* With this novel approach of a chimeric humanized protein, we expect to minimize the recognition by the immune system of the ASNase epitopes and thus reduce its immunogenicity when administered in humans. Furthermore, this concept of "transplantation" of secondary structures is an approach that can be used in a transversal way in the expression of recombinant proteins for different therapeutic purposes.

Effect of *Helicobacter pylori* virulence on progranulin gene expression in gastric tissue of dyspeptic patients.

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Helicobacter pylori is an interesting bacterium, associated with gastritis or ulcer, gastric cancer, or MALT lymphoma. Not all those infected progress to malignancy, attributed to various factors that are still poorly understood. Virulence genes *vacAs1m1*, together with *cagA*, are associated with increased malignancy, and *iceA1* is associated with gastric ulcers. Progranulin (PGRN) is considered as an epithelial growth factor, expressed in wound repair, tissue inflammation, cartilage development, and tumorigenesis, although few studied in gastric lesions despite sharing some mitogenic activation pathways and inflammatory cytokines with *H. pylori.* For this reason, the effect of the virulence of *H. pylori* strains on the expression of the PGRN gene in different states of healthy or injured gastric tissue was studied. PGRN gene expression was determined by qPCR in endoscopically obtained biopsies in 75 infected and 75 uninfected dyspeptic patients from the Region of La Araucanía, who signed the informed consent, prior approval of the ethics committee. Bacteria isolated from Columbia agar supplemented with 7% equine blood and antibiotics (Dent 2%, Oxoid®, UK) and cultured in microaerophilic plants were genetically characterized according to the *ureC, vacA, cagA,* and *iceA* genes by PCR. The levels of progranulin were compared according to the status of gastric tissue determined by endoscopic observation and the infectious status. PGRN was more expressed in erosive (1.56±0.86) lesions, and the lowest value in atrophic (0.65 ± 0.21) and metaplasia (0,89 ± 0,0) lesions ($p<0.05$) and it was accentuated in front of cagA+ or vacAs1m1+ and iceA1+ strains. The most virulent strains negatively affect the genic expression of the protein, being able to stop the potential tissue repair effect of its. Therefore, it is necessary to deepen the effect of *H. pylori* on the genic expression of PGRN and, increase the sample size to consider it as a potential prognostic indicator of gastric infections.

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Towards next-generation colorectal cancer diagnosis: cancer exosomes-derived miRNA for CRISPR/Cas13-based detection.

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Colorectal cancer (CRC) is the third most prevalent cancer with the second-highest mortality rate worldwide. CRC is a heterogeneous disease with multiple risk factors associated, including obesity, smoking, and alcohol. Of total CRC cases, 60% are diagnosed in late stages, where survival can drop to about 10%. Currently, CRC screening programs are based primarily on colonoscopy. However, this approach is invasive and has overall low patient adherence. Therefore, there is a strong incentive for developing molecular-based methods that are minimally invasive and have higher patient adherence. Accordingly, extracellular vesicles known as exosomes act as essential intercellular communication vehicles with a broad cargo, including micro-RNAs (miRNAs). These have been shown as robust candidates for diagnosis, primarily for their protected load of valuable biomarkers. Moreover, cancer-derived exosomes (CEx) play a pivotal role in cancer progression since their cargo is hijacked to promote immunoevasion, tumor progression, and angiogenesis. Accordingly, miRNAs are dysregulated, packed into exosomes and then delivered to target cells. Thus, CRISPR/Cas-based platforms may represent cost-effective, ultrasensitive, specific, and robust clinical detection tools in the presence of potential inhibitors and capable of delivering quantitative and qualitative real-time data for enhanced decision-making to healthcare teams. Thereby, CRISPR/Cas13-based technologies have become a potential strategy for early CRC diagnosis detecting CEx-miRNAs. Furthermore, CRISPR/Cas13-based platforms ease of use, scalability and portability also showcase them as a potential point-of-care (POC) technology for CRC early diagnosis with a reported cost per reaction in a range of 1 USD. Through an extensive review and comparison of current CEx-miRNAs, this study presents two potential CRISPR/Cas13-based methodologies with a proposed panel consisting of four CEx-miRNAs, including miR-126, miR-1290, miR-23a, and miR-940, to streamline novel applications which may deliver a potential early diagnosis and prognosis of CRC, ultimately enhancing patient healthcare and medical teams decision-making processes.

Smoking habit does not affect the biological responses induced by leucocyte and platelet-rich fibrin.

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Leucocyte and platelet rich fibrin (L-PRF) stimulates wound healing in non-smokers and could be an alternative to improve the deleterious effects on tissue repair recognized in smokers. However, the biological effects of L-PRF derived from smokers have not been reported. This study analyzes the kinetics of biomolecules release from L-PRF derived from smokers, non-smokers, and their effect on periodontal cell proliferation and migration as essential biological activities during wound healing. Biomolecules present in L-PRF exudates and conditioned media (LPRF-CM) collected from smokers and non-smokers, were analyzed by Luminex arrays. Periodontal ligament cells obtained from smokers and non-smokers were treated with L-PRF exudates or LPRF-CM to evaluate cell proliferation and migration. A similar biomolecular profile was detected in L-PRF exudates and LPRF-CM from smokers and non-smokers, stimulating periodontal ligament cell proliferation and migration at a comparable degree. L-PRF derived from smokers could be an autologous source of biomolecules to stimulate cell biological activities involved in wound healing in smokers. Clinical trials are required to evaluate the impact of L-PRF on healing responses in smokers.

Symposium IV: Applied Science: the challenge of linking academy and industry

Trampolín Lab: A University program to foster science-based entrepreneurship.

Valdebenito-Godoy, Franklin

Dirección de Innovación y Transferencia Tecnológica (DITT), Vicerrectoría de Investigación y Postgrado, Universidad de La Frontera, Temuco, Chile.

From the establishment of the Chilean Ministry of Science, Technology, Knowledge and Innovation, and the restructuring of the National Agency for Research and Development (ANID), a public policy has been promoted to support the development of science and technology-based entrepreneurship, as an effective instrument for the technology transfer of research results to the community, in addition to constituting an engine to boost the national economy. A science and technology-based entrepreneurship (EBCT), is a startup created from R&D activities carried out within academic, scientific-technological institutions and companies, or together, as a partnership among them (EBCT Study, MCTI, 2020). Specifically, EBCTs emerge as a new and important means of technology transfer through which a research result can be successfully disseminated to the market based on scientific knowledge, also giving young and experienced researchers the possibility to explore new possibilities of professional development. Given the particular nature of these startups originally associated with research projects, it is fundamental to promote a close linkage to the scientific and technological work of our University, as well as a highly specialized institutional support to the researchers/ entrepreneurs who lead this type of initiatives in its initial phases. Due to the above, and given the well-known expertise of the Technology Transfer Unit at the national level regarding the generation of intellectual property strategies, technology transfer processes, associations with the industry, technological management of all R&D projects of our institution, as well as the management of applied research results, makes this Unit the ideal partner to enhance the development and consolidation of EBCTs. In this context, the Innovation Office of the University through its Technology Transfer Unit have designed a specialized program -TRAMPOLÍN LAB- in order to increase the chances of success of scientific endeavors emerging from within our research laboratories. This program aims to promote the generation of EBCTs led mainly by doctoral and postgraduate thesis students, and is based on five fundamental pillars: exploration, training, connection, financing and internationalization.

Low environmental impact and low-cost detergent based on biosurfactants obtained from Antarctic bacteria.

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Biosurfactants are amphipathic molecules composed of two regions, one hydrophilic or polar and the other hydrophobic or non-polar. Biosurfactants are excreted extracellularly by different microorganisms, such as bacteria, fungi, and yeasts. Interest in these products has increased in recent years due to their applications in industry and environmental biotechnology. Microbial biosurfactants are potentially replacing synthetic surfactants in various industrial processes, such as in the production of lubricants, humectants, softeners, dye fixation, emulsions, foaming agents, in the biomedical industry. A current area of interest is the elimination of microbial biofilms generated in salmon young fish culture ponds. In economic terms, it is estimated that around 20% of the production is lost due to deaths caused by diseases generated by pathogenic microorganisms, about US$700 million per year. The objective of this project was to create a technology-based company oriented to the production of a biosurfactant detergent generated by an Antarctic bacterium with microbial biofilm inhibition activity. The bacteria selected for this project were isolated and characterized from the results of the PhD thesis supported by INACH projects Rt_14-15 and Dt_05-15, from the rhizospheric soil of the South Shetland Islands. In this VIU project, the production of biosurfactants was optimized at the reactor scale, in the following parameters: temperature, pH, agitation, and carbon source. The results are under invention protection. The biosurfactant obtained proved to have excellent inhibition activity of microbial biofilm formation and low toxicity, up to one month after its application. Concerning the market for a product such as the one described, the Araucanía Region is the main fish farming area in the country, which gives the project an important advantage due to its geographic proximity to the production centers. In this context, it is important to note that in fish farms, the consumption of detergents and disinfectants throughout the life cycle of the salmon exceeds 1,000 liters per ton of production, which means that salmon young fish producers are very interested.

Development of a cream for topical use with an improved formulation that ensures greater efficacy of photodynamic therapy in the treatment of non-melanoma skin cancer.

León-Garrido, Daniela

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Non-melanoma skin cancer (NMSC) is the most frequent neoplasm worldwide, every year there are 2- 3 million new cases. NMSC frequent lesions such as squamous cell carcinoma precursor lesions can be treated with photodynamic therapy (PDT). PDT is a treatment that involves applying a cream with a photosensitizer on the lesion for a certain time. Then, the lesion is exposed to a specific light that excites the photosensitizer and it reacts with the O2 present in the cell, generating reactive oxygen species (ROS), which causes a cytotoxic effect. This treatment offers advantages over surgery, gold standard treatment, as it is not invasive, it can be applied in large areas of skin or in lesions difficult to access, highlighting above all satisfactory cosmetic results. However, the complete response rate reported to PDT varies, which indicates that there is a group of lesions that are resistant to treatment. Therefore, PDT offers a great opportunity for ambulatory treatment and with important cosmetic results in patients, but it must be enriched to increase the rates of complete response. The aim of this project was to enhance the effectiveness of PDT with methyl aminolevulinate (MAL) as a photosensitizer, improving the photosensitizing cream formulation by adding epigallocatechin gallate (EGCG). Biologically-active compounds (MAL+EGCG) of this formulation were tested in vitro (PDT resistant HSC-1 cells) and as a cream was tested in vivo (squamous cell carcinoma mouse model). According to the in vitro results, the new formulation significantly increased the cytotoxicity of PDT, killing 80% and 100% of resistant cells using EGCG 20 µM and 40 µM, respectively, ($P < 0.05$). Meanwhile in the in vivo model a tendency to decrease the size of the carcinogenic lesions was observed. With this data, an international patent application was filed (WIPO). Furthermore, this research continues through a recently awarded IDeA I+D FONDEF project (ID21I10027).

Early detection and monitoring of pathogenic microorganisms in the salmon industry.

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Chilean salmon industry is constantly growing and possess a market that exceed 4,600 million dollars per year, however, several diseases caused by bacteria and viruses has a considerably negative impact in this production. Diseases such as Piscirickettsiosis, Piscine reovirus infection (PRV), infectious salmon anemia (ISA), Flavobacteriosis, Pancreatic Necrosis Virus (IPN) and Renibacteriosis are some of the most common in the local industry. In this context, rapid and effective diagnosis focused to the prevention of these diseases is a relevant need since it constitutes the first step in addressing this problem. Our project aim to develop an early detection system and a microbiological monitoring service based on third-generation sequencing and bioinformatics which can offers new monitoring tools to the salmonid industry. Our objective is to develop a tool for the monitoring and identification of pathogenic bacteria and viruses applicable in different stages of salmonid production. In this context, we are developing sequencing methods for molecular markers and metagenomics and genomics approaches. This project is based on Nanopore sequencing combined with the development of a customized bioinformatic software for pathogens detection. The expected results of this innovation are to validate our system by the salmonid producers and also be considered as part of the routine quality control carried out on salmonid farms.

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Posters

Exogenous methyl jasmonste application increases growth and photosynthetic pigments in *Vaccinium corymbosum* under toxic conditions of aluminum and manganese in acid soil.

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Acid soils (pH ≤ 5.5) represent 30-40% of the cultivated area in the world; however, soil acidity can favor phytotoxicity by aluminum (Al) and manganese (Mn). *Vaccinium corymbosum* is a species of great agroeconomic interest affected by these conditions. Therefore, it is necessary to find agronomic strategies to reduce the damage caused by aluminum and manganese toxicity, such as using exogenous plant hormones like methyl jasmonate (MeJA). Therefore, the objective was to evaluate the effect of the exogenous application of MeJA on the development of *V. corymbosum* exposed to Al- and Mn-induced stress. For this purpose, the cultivar Legacy growing under field conditions was exposed to an excess of Al (86% saturation) and Mn (240 mg kg-1), with and without MeJA (10 µM) application. During harvest season, leaves were analyzed, growth rate (GR), Al and Mn concentrations, and photosynthetic parameters such as CO2 assimilation, stomatal conductance, transpiration, and photosynthetic pigment were measured. Our results showed that exogenous MeJA application increased GR in Al and Mn treatments by 10 and 40%, respectively, compared to treatments without MeJA. There were no significant differences between +MeJa and -MeJa treatments exposed to Al, Mn, or Al+Mn toxic conditions regarding plant uptake of these metals. On the other hand, the presence of MeJa increased the concentration of photosynthetic pigments in leaves in the combined Al and Mn stress treatment. Chlorophyll a, b, and a + b increased by 15-25% and Β-carotene by 40%. MeJA did not affect other photosynthetic parameters such as CO2 assimilation, stomatal conductance, or transpiration. In conclusion, MeJA application could be useful as an agricultural tool in *V. corymbosum* to mitigate Mn and Al stress damage, increasing GR and photosynthetic pigments.

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Protective effect of polyphenols found in Pinot noir pomace extract against cytotoxic effect of polycyclic aromatic hydrocarbons in vitro.

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Polycyclic Aromatic Hydrocarbons (PAH) are pollutants primarily generated by coal combustion or biomass burning and found in the air. PAH produced by firewood pollution in Temuco, Chile, are mainly composed of Fenantreno (35 - 45%), Fluoranteno (11 - 15%) and Pyrene (9 -12%). PAH are positively correlated with ROS production in endothelial cells, causing their dysfunction and allowing the development of cardiovascular diseases. Phenolic compounds are naturally found in the plant kingdom, and their presence in agri-food processing wastes has led to increased recovery interest, encouraging the valuation of high added value by-products. The grape pomace (GP) undergoes incomplete extraction during the winemaking process, increasing its phenolic components, which are beneficial for human health due to both, their antioxidant activity and antimicrobial, antiviral and anti-inflammatory properties. The aim of the present study was to evaluate the effectiveness of the extract generated from Pinot noir pomace (EGP) as a natural product in the protection of human endothelial cells against the cytotoxic effect of PAH. The molecular profile of EGP was evaluated through HPLC and its antioxidant capacity through ABTS and ORAC. The effect of PAH and EGP on the viability of HUVEC cells was evaluated through MTS assay. Subsequently, 400 µg / mL of EGP was used to evaluate the protective effect on HUVEC cells stimulated with HPA. Analysis revealed the presence of five glycosylated anthocyanins and nine low molecular weight polyphenols. Molecular coupling indicated that cyanidin-3-glucoside (-9.2 kcal/mol) and quercetin (-9.6 kcal/mol) showed the highest affinities for the Nrf2 binding site in the Keap1 protein, suggesting a possible competition with this transcription factor. HUVEC cells were exposed to increasing concentrations of PAH diluted in DMSO in a ratio of 3: 1: 1 (10µM-200µM). The viability evaluated through the MTS assay showed that 150 µM PAH was sufficient to reduce viability by 75% (p 0.0001). When cells were pre-treated with 400 µg / mL of EGP, 150 µM of PAH did not exert cell death (80% viability). In summary, our results suggest that the polyphenolic components found in EGP could have a beneficial effect as a protective agent in individuals living in contaminated areas with PAH, such as Temuco city.

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Reduction of phytotoxic effect of manganese in horticultural crops by extremophiles microorganisms.

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Soil can be strongly altered by anthropogenic factors like industrial activities and wrong agricultural practices, which can change the natural concentrations of minerals elements in soil causing contamination. The manganese (Mn), can be found in soils in three oxidation states: Mn (II), Mn (III), and Mn (IV), being Mn (II) state which of uptake by plants. High concentrations of Mn produce physiological damage in plants as chlorosis, necrotic spots, as well as decrease of photosynthetic rate. An ecological solution for this problem could be use of rhizospheric microorganisms that could reduce the physiological damage produced by high metals concentration. The aim of this work was to study the phytotoxic effect of high concentration of Manganese in Lactuca sativa plants inoculated with extremophiles microorganism. The Manganese tolerance test was made to select resistant microorganism and select as inoculant. Chlorophyll A, B, total Chlorophyll, lipid peroxidation and hydrolysis of fluorescein di-acetate (FDA) were evaluated. We found four fungi tolerant to high levels of Manganese (*Plectosphaerella cucumeris, Chaetonium cupreum, Fusarium equiseti, Penicillium citreosulfuratum*) and one bacterium (*Bacillus megaterium*). Treatments with high levels of Mn (2000 mg/L) showed a decrease in both chlorophyll and biological activities, as well as an increase in lipid peroxidation of L. sativa plants. The inoculation with selected fungi and bacterium from extremophiles environments reduced the toxic effect of manganese at the physiological level, so they could be used as biotechnological tools in the bioremediation of soils with high concentrations of manganese.

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Is the trade-off between stress tolerance and photosynthetic capacity the predominant response under the extreme environment of Atacama Desert? Searching for outlier species with special traits for future application in plant breeding.

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A negative correlation or "trade-off" between allocated resources for primary productivity and stress tolerance is generally observed in nature. In this context, the search for "outlier" plants breaking this trend is an interesting approach to find new physiological or molecular mechanisms for plant breeding purposes. Hypothetically, outlier plants living under extreme environments present a distinctive arrangement of physiological functions that favor stress tolerance mechanisms without jeopardizing investment allocation into productivity. Here, we explored this trade-off, analyzing fifteen plant species for desiccation tolerance, freezing tolerance, and photosynthetic capacity, under the extreme environment of the Atacama Desert. Most of the studied species followed the trade-off tendency; however, we found one outlier species, Prosopis tamarugo. To understand the mechanisms involved in this atypical response, three species from the Prosopis genus with contrasting responses to the trade-off were studied more deeply. Physiological, biochemical, and metabolomic analyses at the leaf level, suggest that the outlier response observed in P. tamarugo is multifactorial, including efficient photochemistry and photoprotection, based on the synthesis of higher contents of chlorophylls and carotenoids, along with the synthesis of tryptophan derivate, and complex antioxidant metabolites, favoring its photosynthetic capacity without compromise its abiotic stress tolerance. Our results have shown marked differences in the strategies employed by congeneric species which can represent adaptive advantages to grow in an extreme environment such as the Atacama Desert. Moreover, the mechanisms identified could be very useful as target traits for selection in tree breeding or biotechnology purposes and could help to improve our strategies for the conservation of this endangered species

Effect of soluble sugars on apoplastic osmolarity of antarctic vascular plants.

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The accumulation of sugars is a common cryoprotective mechanism in several plant species. Among those we find Antarctic vascular plants: *Deschampsia antarctica* and *Colobanthus quitensis.* However, it is not clear which of all soluble sugars accumulated by these species is related to apoplastic solutes osmolarity. So, we decided to compare the freezing point (FP) values of leaf tissue and the specific amounts of the most abundant soluble sugars. For which the freezing point was evaluated by means of a thermal analysis; and the amounts of sugars by HPTLC quantification: the measurements were performed on plants from laboratory with different temperature regimes (8/0ºC; 8/6ºC; 14/0ºC and 14/6ºC), and from Antarctica under natural conditions and within passive heating systems. In *D. antarctica* the most abundant sugar was sucrose with values ranging between 120 & 50 mg/g DW, which seems to be inversely related to FP values ranging between -20ºC & -17.9ºC in this species, particularly in laboratory plants; while in *C. quitensis* the most abundant sugars are stachyose and raffinose with values ranging between 85 & 34 mg/g DW and 66 & 27 mg/g DW respectively, and the latter seems to be inversely related to FP values ranging between -18.2ºC & -16.7ºC. Our results suggest that sucrose in D. antarctica and raffinose in *C. quitensis* are mainly responsible for the osmolarity of the apoplastic fluids of these species.

Ethanolic extract of propolis modulates autophagy related microRNAs in osteoarthritis chondrocytes.

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Osteoarthritis (OA) is a degenerative and multifactorial joint disease and aging is the most important risk of factor. Autophagy is a mechanism that maintains cell homeostasis in cartilage and is widely affected by aging, a phenomenon that is correlated with cell death and OA. During the development of OA, both environmental and genetic factors influence chondrocyte biology through epigenetic regulation and these modifications could represent a therapeutic opportunity for OA, a currently intractable disease. On the other hand, miRNAs have emerged as important regulators of autophagy and miRNA-based therapies have been analyzed in preclinical models. In addition, it has been described that the ethanolic extract of propolis (EEP) produces beneficial effects such as a regulation of autophagy proteins and markers of healthy cartilage in an *in vitro* model of OA. The present study evaluated if the effect of EEP on autophagy proteins could be mediated through an epigenetic mechanism. By means of an in-silico analysis, five miRNAs related to three genes of the autophagy pathway (AKT1, ATG5 and LC3) were identified in the New Zealand rabbit species *Oryctolagus cuniculus:* miR-125b-5p; miR-185-5p; miR-19a-3p; miR-181a-5p and miR-335-5p. Its expression was evaluated by RT-qPCR in chondrocytes stimulated with IL1B (control) and in chondrocytes stimulated with IL1B and treated with EEP (treated group). Treatment with EEP in chondrocytes with OA generates a significant change in the expression of three of the five miRNAs evaluated. There was a decrease in the expression of miR-181a and miR-125b, which regulates the expression of ATG5 and AKT1, respectively, and an increase in the expression of miR-185 that regulates the expression of AKT1. Our results show that EEP could regulate the expression of microRNAs. However, these results must be confirmed by functional studies.

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Effect of inhibition of protein synthesis and phosphorylation on bovine oocyte activation and embryonic development.

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In equine and porcine to improve efficiency of oocyte activation, combined use of inhibitors of protein-synthesis and phosphorylation has been described; however, there are not enough studies in bovines. In present study we compared the effect of synthesis-inhibitors (ANY and CHX) and protein-phosphorylation (DMAP) and its combination (ANY; ANY+CHX; ANY+DMAP; ANY+CHX+DMAP; CHX+DMAP) on parthenogenetic activation of bovine oocytes, assessing the embryonic development up to blastocyst stage under stereo microscope and expression and status of maturation-promoting-factor (MPF) components (cyclin-B1, CDK1, p-CDK1Thr161 and p-CDK1Thr14-Tyr15) and ERK1/2 pathway by immunodetection at 4 hours-post-activation. Results did not show differences between treatments in cleavage rate (91.6-98.6%) and blastocyst rate (37.4-50.9%). Moreover, the treatments did not modify the level expression of cyclin-B1 and total CDK1, nor p-CDK1^{Thr161} and p-CDK1$^{Thr14\text{-}Tyr15}$. However, decrease expression of ERK1/2 protein was observed in oocytes treated with ANY and ANY+CHX, compared to ANY+DMAP, ANY+CHX+DMAP and CHX+DMAP treatments ($p<0.05$). Besides, decrease p-ERK1/2 was observed in ANY+DMAP, ANY+CHX+DMAP and CHX+DMAP, compared to ANY and ANY+CHX ($p<0.05$).
The results indicate that the activation of bovine oocytes with treatments used in the present experimental design does not occur by decrease of cyclin-B1. Also, since the levels of activating (p-CDK1^{Thr161}) and inhibitory (p-CDK1Thr14-^{Tyr15}) phosphorylation of MPF were observed in balance, we infer that MPF is present in an inactive form (preMPF). Moreover, protein-synthesis-inhibitors alone (ANY) or in combination (ANY+CHX), generated decrease of ERK1/2 on a similar level. While protein phosphorylation inhibitor (DMAP) combined with ANY, CHX or both, decreased phosphorylation of MAPK, compared to treatments with protein synthesis inhibitors. In conclusion, treatments used favors the embryonic development and promote the activation of bovine oocytes in ERK1/2-dependent manner. Futures analyses of gene expression are necessary to assess the treatments effect on embryonic quality.

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Effect of pre-treatment of frozen stallion sperm with Triton X-100 on destabilization of plasma, acrosomal membranes, sperm membrane fluidity and DNA fragmentation.

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In intracytoplasmic sperm injection (ICSI), complete spermatozoa is injected into the oocyte containing all its membranes and also the acrosomal content with enzymes such as lizosim, acrosin, among others. Membranes and acrosomal content have been shown to have a detrimental effect on the oocyte, which is related to the size of spermatozoa. Removal of acrosomal content can be achieved by different treatments including pronase, lysolecithin (LL), and triton X-100. The objective of this research was to evaluate the effect of pretreatment in equine spermatozoa with newt X-100 on plasma and acrosomal membrane destabilization, membrane fluidity and DNA fragmentation to be used as a novel treatment to improve the efficiency of ICSI in equine species. Semen samples were thawed by incubating at 37°C for 30 seconds, permeabilization with triton X-100 were performed at different concentrations with vortex for 1 min, the integrity of plasma membrane was analyzed for each treatment. Lastly, acrosomal membrane destabilization, membrane fluidity and DNA fragmentation were analyzed by flow cytometry. Incubation of equine sperm at different concentrations of LL significantly decreased the integrity of plasma membrane among the different concentrations ($p < 0.05$), while only slight damage to the acrosomal membrane was observed. Both membrane fluidity and DNA fragmentation evidenced an increase at the highest concentrations of the evaluated treatments. Pretreatment of equine spermatozoa with Triton X-100 effectively permeabilizes plasma membrane, although an effect on acrosomal membrane is not evidenced. Incubation of equine sperm with Triton X-100 also increases membrane fluidity, confirming that this treatment could positively impact on the efficiency of ICSI in horses.

Effect of cholestanol and cholesterol-loaded-cyclodextrin on physiology and in vitro capacitation of stallion sperm after cryopreservation.

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Cryopreservation of stallion semen is inefficient and does not shows the positive results described for other species. This is because of the high susceptibility of stallion sperm to the freezing damage that generates oxidative stress and plasma membrane injury, resulting in DNA fragmentation and cell death. These data suggest the need of finding new strategies for sperm cryopreservation, which could improve the efficiency of the currently used technique in stallion semen cryopreservation by preventing the membrane damage and cell death. The present study aimed to evaluate the effect of adding membrane stabilizers to the freezing medium and assess the quality and in vitro capacitation of stallion sperm after thawing. Samples from three stallions frozen with membrane stabilizers (cholesterol-loaded cyclodextrin and cholestanol-loaded cyclodextrin) were evaluated in two experiments: i) sperm quality analysis after thawed, and ii) sperm quality and functional analysis after 4 h of post-thawed incubation in capacitation conditions. Plasma membrane integrity, mitochondrial membrane potential, membrane lipid disorder, intracellular $^{Ca2+}$, tyrosine phosphorylation, acrosome reaction, DNA damage, sperm motility, and binding to the zona pellucida were assessed. The results showed that cholesterol loaded cyclodextrin was the stabilizer that best controlled the membrane disruption and post-thaw cell damage. In addition, this stabilizer allowed to obtain in vitro capacitated sperm showing higher plasma membrane integrity, mitochondrial membrane potential, sperm motility, number of spermatozoa bound to the zona pellucida and better response to in vitro capacitation conditions.

Genetic diversity of EPIYA motifs on Helicobacter pylori carrying CagA from dyspeptic patients of Southern Chile.

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The human pathogen *Helicobacter pylori* (Hp) displays extensive genetic diversity. We obtained gastric biopsies of 26 dyspeptic patients infected with Hp carrying CagA virulence gene and analyzed their genetic diversity of EPIYA motifs in this virulence factor with association of clinical outcome lesions. Gene analyses were performed with MEGA V 7. Statistical analysis was performed with Graph Pad Prism and chi-square test was used to differences between lesions, presence of EPIYAs, age, sex, ethnic population, degree of significance in $p < 0.05$. Genetic differentiation of this motifs was performed with DNAspv5.10, evaluating (Fst), Gene flow (Nm) and Tajima test. The sequences were observing the differences in the regions corresponding to EPIYA, we obtained 21 typical Western sequences, but five sequences that presented mutations in the region before EPIYA-A with East sequence characteristics. The alignment of amino acid sequences confirmed that EPIYA motifs mutated had evidence of mutations. Comparing the divergence of EPIYA(ABC, ABCC, ABCCC) were no significant differences between the sample studied ($p=0.732$) in relation to the severity of clinical lesions ($p=0.931$), in relation to the sex of the patients ($p=0.264$), in relation to the population ($p=0.477$), but presented significant differences in the age of the patients, prevalence of adult patients ($p=0.03$), and in relation to clinical lesions and EPIYA that have undergoing mutation ($p= 0.0006$), demonstrating that strains with mutated EPIYA have a greater worsening of the lesions. The values of Fst and Nm were 0.31485 and 1.09. In addition, we obtained a negative value for the Tajima's test D: -0.611, where it leads to assume recent population expansion and is indicative of low frequency of mutations. These results provide addition to further studies with a larger sample amount, characteristics for protein modulation and phylogenetic studies to know how and where this bacterium is migrating from and its clinical implications.

Use of the Fructosiltransferase of *Aspergillus oryzae* N74 as part of a conjugate for indirect quantification of DNA with glucose as response.

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In the search of novel alternatives for the detection and quantitation of nucleic acids this project proposes a system that involves DNA fragment amplification through a loop-mediated isothermal amplification (LAMP) reaction and its indirect quantitation by measuring the glucose released with a thermostable invertase. This system involves the generation of a conjugate carrying the recombinant enzyme fructosyltransferase (FTase) from *Aspergillus oryzae* N74 attached to a magnetic nanoparticle by means of complementary DNA fragments, that after interacted with the target DNA fragment allows the release of the recombinant FTase. The free enzyme catalyzes hydrolysis of glucose, which can be detected with a simple quantification method such as the glucose oxidase/peroxidase reaction or a conventional glucometer. As proof of concept, this work used the gene for the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS), commonly used at the Institute of Inborn Errors of Metabolism for Morquio's disease research. This gene was initially detected and quantified by real-time PCR (qPCR) at concentrations between 0.1 and 5000 ng/µL. Subsequently, the gene was detected and quantified using the conjugate carrying the FTase enzyme. The results showed that amplification with LAMP allows the detection of DNA by fluorescence and that the amplification product can be integrated to the conjugate for the quantification of the DNA. The binding of an invertase enzyme to the conjugate and the construction of specific oligonucleotides for the target region made it possible to establish a directly proportional relationship between glucose release and DNA concentration between 0.1 and 1000 ng/µL. The use of recombinant FTase enzyme from *Aspergillus oryzae* N74 allowed the hydrolysis reaction to be carried out in concentrations between 125 and 1000 mM of sucrose, with 500 mM being the concentration that showed the best performance for DNA quantitation. Since this method does not require sophisticated equipment for its execution, it constitutes an important tool for the design of nucleic acid identification and quantitation methods that can be adaptable to research and diagnostic laboratories with different degrees of complexity.

Fasciola hepatica population structure analysis by microsatellite PCR.

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Fasciola hepatica is a trematode of the Phylum Platyhelminthes globally distributed on all continents except Antarctica. It seriously affects livestock, impacting in public health and causing significant productive and economic losses derived from the clinical syndrome and the liver condemnation. The population structure of *F. hepatica* is not well known and there are several characteristics that influence: complex life cycle, clonal expansion in the snail intermediate host and self and cross fertilization and parthenogenesis. Whilst, microsatellite markers have been used for population genetic and mapping quantitative trait loci, among other applications. The aim of this work was determinate the genetic structure population by using microsatellite markers in a *F. hepatica* population. We use genomic DNA from 53 flukes (10 bovine hosts), sourced from the Nueva Imperial city abattoir, Region of La Araucanía, Chile. The hosts were georeferenced through the DIIO (official individual identification device), determining 5 locations. A polymorphic locus in the *F hepatica* genome was amplified with one microsatellite primer pair (Fh_2). The molecular weight of the alleles was measured using Gel Analyzer v 19.1 program. The genetic population analysis was performed using the Popgene software v 1.32. A total of 15 different alleles were found. The observed heterozygosity (H_o) ranged from 0.25 to 1.00 between the flukes of each bovine. Nei's expected heterozygosity of all populations was 0.849 and genetic structure evidenced by F^{ST} was 0.176. The highest genetic distance was observed between Máfil and Lautaro, while the greatest Nei's genetic identity was obtained in the Máfil and Villarrica locations. These results were similar to previously published microsatellite data. This microsatellite marker provide insight into geographically distinct isolates. The projections of the work include increasing the number of samples and origin sites, as well as using of more microsatellite primers. This strategy would allow a more comprehensive study of the population structure of *F. hepatica* in our country.

Effects of Four Lipid Metabolism-Related Genetic Variants on Body Composition Improvements after 12 Weeks of High-Intensity Interval Training and Dietary Energy Restriction in Overweight/Obese Adult Women.

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Polymorphisms in lipid metabolism-related genes have been associated with obesity and body composition, but these have been scarcely described concerning the magnitude of the response to exercise interventions in the overweight/obese population. To evaluate the association of PLIN1 (rs1052700 and rs2304795), LPL (rs283), ADRB3 (rs4994) polymorphisms with high and low responders to fat mass reduction after 12 weeks of high-intensity interval training (HIIT) and dietary energy restriction in overweight/obese adult women, and to evaluate the effect of these genetic variants on body composition changes. Forty-three unrelated overweight/obese adult women were incorporated and genotyped, of which 30 women (age= 27.4 ± 7.9 years; BMI= 29.9 ± 3.3 kg/m2) successfully completed the 12-week supervised HIIT program plus an individually prescribed home hypocaloric diet. An association was observed between the PLIN1 rs1052700 polymorphism with high and low responders ($\chi 2$=8.138; 2 df; P=0.01). Moreover, after the intervention, the carriers of TT genotype of PLIN1 rs1052700 as compared to AA and AT genotypes showed a greater reduction in absolute fat mass (Δ: - 5.1 ± 1.8 vs. -1.8 ± 1.4 vs. -2.1 ± 2.3 kg; P=0.04). The effect size of this fat mass reduction between TT and AT genotypes was a mean difference of -3.01 kg [95%IC -4.88 – -1.1], and between TT and AA genotypes was -3.29 kg [95%IC -4.86 – -1.65]. No differences were observed for other polymorphisms investigated. These results suggest that the rs1052700 (14995A>T) polymorphism of the PLIN1 gene is associated with a differential response to fat mass reduction after a 12-week intervention in overweight/obese adult women. In addition, women with the TT genotype of this genetic variant showed greater changes in fat mass than AA and AT genotypes. However, further studies are needed to confirm these findings.

Glucokinase 1 from *Phytophthora infestans* is not a true glucokinase.

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Phytophthora infestans is the causative agent of late blight disease. According to its sequence, the [PITG_06016] gene encodes for glucokinase 1 from *P. infestans* (PiGlcK-1), an enzyme belonging to the group of glucokinases A of the large Hexokinase family. Its expression in the different infectious structures is the highest compared to the other 6 presumed glucokinases present in this phytopathogen. In this study, we propose that PiGlcK-1 could be phosphorylating other sugars present during the establishment of infection and not just glucose.To test this hypothesis, we cloned, overexpressed, purified and biochemically characterized this enzyme. We found that the purified recombinant protein has a KM of 0.66 mM and 2.17 mM for glucose and ATP, respectively. PiGlcK-1 is able to phosphorylate fructose by 48% with respect to glucose, it uses ADP and pyrophosphate (PPi) as phosphoryl group donors. These molecules are 50% and 11% less effective than ATP, respectively. Furthermore, PiGlcK-1 is activated by PPi 3.6 or 2.5 times when the phosphoryl donor is ATP or ADP, respectively. These results reveal that PiGlcK-1 is not a true glucokinase, and suggest that its classification should be revised. Furthermore, we propose that PiGlck-1 is a key enzyme in the metabolism of P. infestans that could be playing an important role in the use of sugars available during the first stage of infection, as well as in the necrotrophic stage. These findings represent an important advance in the understanding of the metabolism of this phytopathogen and contribute to the search for a truly effective and safe solution to the threat posed by late blight on potato and tomato crops.

Keywords: *Phytophthora infestans,* glucokinase, recombinant protein, sugars, enzyme activation.

MicroRNAs hsa-miR-618 and hsa-miR-297 Might Modulate the Pleiotropic Effects Exerted by Statins in Endothelial Cells through the Inhibition of ROCK2 Kinase: *in-silico* Approach.

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Several studies show that statin therapy improves endothelial function by cholesterol-independent mechanisms called "pleiotropic effects." These are due to the inhibition of the RhoA/ROCK kinase pathway, its inhibition being an attractive atheroprotective treatment. In addition, recent work has shown that microRNAs, posttranscriptional regulators of gene expression, can affect the response of statins and their efficacy. For this reason, the objective of this study was to identify by bioinformatic analysis possible new microRNAs that could modulate the pleiotropic effects exerted by statins through the inhibition of ROCK kinases. A bioinformatic study was performed in which the differential expression of miRNAs in endothelial cells was compared under two conditions: Control and treated with simvastatin at 10 µM for 24 h, using a microarray. Seven miRNAs were differentially expressed, three up and four down. Within the up group, the miRNAs hsa-miR-618 and hsa-miR-297 present as a predicted target to ROCK2 kinase. Also, functional and enriched pathway analysis showed an association with mechanisms associated with atheroprotective effects. This work shows an *in-silico* approach of how posttranscriptional regulation mediated by miRNAs could modulate the pleiotropic effects exerted by statins on endothelial cells, through the inhibition of ROCK2 kinase and its effects.

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Atorvastatin differentially modulates the expression of lncRNAs associated with cholesterol metabolism in hypercholesterolemic Chilean individuals.

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Hypercholesterolemia is considered an important risk factor for cardiovascular diseases. The use of statins is the treatment of choice in hypercholesterolemic patients. However, the wide interindividual variability observed in response to this drug still needs further elucidation. Nowadays, long non-coding RNA (lncRNAs) associated to cholesterol metabolism are known and these molecules could be involved in the variability of response. The objective of this research was to evaluate the atorvastatin effect on three lncRNAs (RP1-13D10.2, MANTIS and lncHR1) associated with genes involved in cholesterol metabolism, as predictors of lipid-lowering therapy. Twenty hypercholesterolemic patients, who were treated during four weeks with atorvastatin 20 mg/day, were included in the study. The lipid profile was determined before and after drug administration using conventional assays. The lncRNAs expression in leucocytes of peripheral blood was determined by qPCR. Atorvastatin diminished the levels of total cholesterol, LDL-C y TC/HDL-C ($p<0.0001$). Besides, atorvastatin diminished the lncHR1 expression ($p<0.0001$) in hypercholesterolemic subjects after treatment. However, lncRNAs RP1-13D10.2 ($p=0.6621$) and MANTIS ($p=0.0623$) did not show significant differences after treatment. These results indicate that atorvastatin modulates differentially the expression of lncRNAs associated to cholesterol metabolism, suggesting that these molecules play a role in the variability of response to this drug.

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Characterization of two plasminogen-binding proteins in Trypanosoma evansi. A protein guanine nucleotide-binding protein similar to the beta subunit and elongation factor 1-α.

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Trypanosoma evansi is a widely distributed hemoflagellate parasite of veterinary importance that infects a variety of mammals, including horses, mules, camels, buffalo, and cattle. It is the causal agent of a trypanosomiasis known as Surra that produces epidemics of great economic importance in Africa, Asia and South America. The main pathology includes an enlarged spleen with hypertrophy of lymphoid follicles, congested lungs, neuronal degeneration and meningoencephalitis, where the migration of parasites from the blood to the tissues is essential. Most cells, including pathogens, use various strategies for tissue invasion, such as the expression of surface receptors to bind plasminogen or plasmin. In this study, we report that guanine nucleotide-binding protein, a beta subunit-like protein (TRACK), and elongation factor 1-α (EF-1α) are plasminogen-binding proteins in *T. evansi,* two proteins that participate in various cellular processes. TRACK has been reported to be analogous to LACK (Leishmania activated kinase C homologous receptor) which participates in the plasminogen binding process in *L. mexicana* and induces a strong immune reaction in the mammalian host against infection. EF-1 alpha, for its part, is a ubiquitous multifunctional and highly conserved protein that affects a large number of cellular processes, including protein degradation and the organization of the cellular cytoskeleton. In addition to these functions, plasminogen binding capacity represents a novel function for these proteins. This function by TRACK and EF-1α could be important in the *T. evansi*-interaction with the host cell and could constitute an interesting target for the development of future drugs.

PAHs exposure triggers inflammation and endothelial dysfunction in BALB/c mice.

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Particulate matter in air pollution is partly composed of Polycyclic Aromatic Hydrocarbons (PAHs). Even though PAHs exposure has emerged as an important risk factor for cardiovascular disease, the mechanisms by which these compounds contribute to increased cardiovascular risk have not been fully explored. We aimed to evaluate the effects of PAHs exposure on cardiovascular disease using a murine model comprised of twenty male BALB/c mice separated into controls and three groups exposed to a mixture of Phenanthrene, Fluoranthene, and Pyrene PAHs using three different concentrations. We determined serum levels of inflammatory cytokines and gene expression of adhesion molecules located on endothelial cells along with inflammatory markers related to PAHs exposure in aortic tissue. Finally, ICAM-1 and VCAM-1 protein levels were assessed. Data showed significant differences in serum IL-6 and IFN-γ. In gene expression, we observed significant differences for ICAM-1, VCAM-1, and E-Selectin. Our results suggest that Phenanthrene, Fluoranthene, and Pyrene stimulate serum inflammatory markers and endothelial dysfunction in the murine model studied, both relevant features associated with cardiovascular disease.

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Extracellular microRNAs as potential biomarkers to evaluate the lipid-lowering response to atorvastatin in hypercholesterolemic subjects.

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MicroRNAs (miRNAs) play an important role in the regulation of several biochemical mechanisms, cell proliferation and other cellular mechanisms in the human body. The level of microRNAs in blood and other body fluids (urine, saliva, sweat) increases because of altered pathophysiological mechanisms and tissue insult. Moreover, the stability and specificity of miRNAs make them ideal candidates for circulating biomarkers in several diseases, including cardiovascular diseases. In the present study, we evaluated plasma miRNAs in response to the lipid-lowering drug atorvastatin in patients with hypercholesterolemia (HC) and controls. The miRNAs were selected based on previous data reported by our group and by employing bioinformatics tools to identify 10 miRNAs related to cholesterol metabolism and statin response genes. Following miRNA identification, we determined plasma levels of miRNA-17-5p, miRNA-30c-5p, miRNA-24-3p, miRNA-33a-5p, miRNA-33b-5p, miRNA-29a-3p, miRNA-29b-3p, miRNA-454-3p, miRNA-590-3p and miRNA-27a-3p in 20 HC patients before and after 1 month of 20 mg/day atorvastatin treatment, evaluating the same miRNA set in a group of 20 healthy subjects, and employing qRT-PCR to determine differential miRNAs expression. HC individuals showed significant overexpression of miRNA-30c-5p and miRNA-29b-3p vs. NL ($p = 0.0008$ and $p = 0.0001$, respectively). Once cholesterol-lowering treatment was concluded, HC individuals showed a substantial increase of three extracellular miRNAs (miRNA-24-3p, miRNA-590, and miRNA-33b-5p), the latter elevated more than 37-fold ($p = 0.0082$). Our data suggest that circulating miRNA-30c-5p and miRNA-29b-3p are associated with hypercholesterolemia. Furthermore, atorvastatin induces a strong elevation of miRNA-33b-5p levels in HC individuals, which could indicate an important function that this miRNA may exert upon atorvastatin therapy. Additional studies are necessary to clarify the role of these circulating miRNAs in statin treatment.

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Circular RNAs associated with the expression profile of dysregulated microRNAs in heart failure: *in silico* analysis.

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Cardiovascular diseases (CVDs) are health problems facing the current panorama due to their high mortality rate worldwide. Our country has a similar situation, with CVDs being the leading cause of death with 27% of deaths. Within these diseases, hypertensive, ischemic and cerebrovascular diseases are dominant in our population, which predispose to the development of heart failure. Various studies have reported the role of miRNAs and circRNAs in various diseases, and their regulatory potential over other RNA species. This role has been described through three main mechanisms, functioning as a modulator of the action of miRNAs and protein mechanisms, as well as a modifier of gene expression. One of the mechanisms described refers to the ability of circRNAs to function as a miRNA sponge, capturing through MRE sites and reducing their availability, modulating and influencing the expression of target mRNAs. RNAseq data obtained from public repositories were analyzed using find_circ, CIRI2 and CIRIquant to search for circRNAs, and ShortCat to search for miRNAs. Differential expression was analyzed using DESeq2. CircRNA-miRNA pairs were analyzed for complementarity using RNAhybrid and generating networks with CytoScape. Finally, the data were subjected to an enrichment analysis using enrichR. A total of 9 circRNAs and 12 differentially expressed miRNAs were obtained, whose parental genes or targets are involved in molecular pathways linked to heart failure and associated processes, such as cardiac hypertrophy or remodeling. There is theoretically a relationship between the expression profiles of circRNAs and miRNAs, seeing the described sponge mechanism in action, which would be causing an effect on the gene expression of cardiac tissues in subjects with heart failure.

Epigenetics - the missing link between high blood pressure and periodontitis. A bioinformatics approach.

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Cardiovascular diseases are the leading cause of death worldwide and arterial hypertension is recognized as a cardiovascular risk factor, responsible for a high cardiovascular morbidity and mortality. Arterial hypertension can be considered as the result of an inflammatory process with remodeling and thickening of vascular walls, to which an immune response is associated. Several authors have scientifically attempted to demonstrate the established relationship among oral disease, inflammation, and the development of systemic diseases. The existence of an association between periodontitis and hypertension has been a controversial issue, since the underlying pathophysiological processes and inflammatory mechanisms are unknown. Common to both diseases, periodontitis being a chronic inflammatory disease that affects the interface of the teeth and surrounding tissues. However, the most likely explanation for understanding this association is related to low-grade chronic inflammation. An initial track in the study of the relationship between the aforementioned pathologies is the possibility of an epigenetic influence, mediated by microRNAs. Thus, by use of bioinformatics tools, we identified 13 miRs common to both pathologies: hsa-miR-100-5p, hsa-miR-21-5p, hsa-miR-34a-5p, hsa-miR-146a-5p, hsa-miR-26b-5p, hsa-miR-126-3p, hsa-181a-5p, hsa-miR-15b-5p, hsa-miR-223-5p, hsa-miR-16-5p, hsa-miR-30a-5p, hsa-miR-17-5p and hsa-miR-155-5p that were reported in both arterial hypertension and periodontitis, out of a total of 59 miRs reported related to arterial hypertension and 79 miRs reported related to periodontitis. The role of these microRNAs must be determined in experimental studies.

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FASTKD4/TBRG4: An *in silico* and molecular insight to a novel cancer related gene.

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Fas-Activated Serine/Threonine kinase (FASTK), comprise a family protein identified as mitochondrial RNA homeostasis regulator. Within this family, FASTK domain-containing protein 4 (FASKTD4) or Transforming growth factor β (TBRG4) has been studied and demonstrated its influence on mitochondrial mRNA stability and its contribution to tumorigenesis. Nevertheless, a lack of information related to its structural function and the absence of a possible mechanism of action hinders a proper understanding. We aimed to characterize TBRG4 structure comparing different methodologies and evidencing possible modifications pointing to its activity. *In silico* approaches, such as, homology and de novo modeling, molecular dynamics (MD) and structural characterization of TBRG4 residues sequence has been performed, using 30 ns for MD. RMSD and conformational changes has been identified and described. *In silico* phosphorylation of selected residues has been proposed and related with WT structure. We characterize TBRG4 models obtained by two methodologies: I-Tasser structure modeling and AlphaFold structure database. AlphaFold performed the most related model to TBRG4 residues sequence and secondary structure, being possible to use AlphaFold as our WT model. This model allowed to make a proper description and characterization of three well-formed domains named FAST_1, FAST_2, and RAP, highlighting 82 residues as possible targets of phosphorylation (Serines, Threonines, and Tyrosines). Only serine 553 has been correlated with other studies as active phosphorylated residues. *In silico* mutagenesis has been performed to identify structural changes of TBRG4, showing 2 conformations open and closed. Therefore, we conclude serine relevance in conformational state, changing into an open and closed conformation, allowing next studies focused in the identification of possible target and the suggestion of possible mechanism of action.

Airborne bacterial composition from public waiting rooms of medical centers in Temuco city.

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Indoor bioaerosols are considered indicators of air pollution and can play a critical role in public health. Studies have shown that bioaerosols include microorganisms, such as bacteria, and fungi. Despite the strict and standardized biocontrol protocols in medical centers (clinics and hospitals), a considerable abundance and diversity of microorganisms are present. In this context, there is a wide range of potentially pathogenic airborne microorganisms associated with nosocomial infections in medical centers, which are categorized as opportunistic pathogens that frequently cause respiratory infections in immunosuppressed patients. Therefore, due to the flow and interactions between patients and medical staff, "public waiting rooms" (PWR) in medical centers represent a particular area that could harbor and exchange large amounts of potentially respiratory pathogens. In this study, the main objective was to determine the bacterial composition in air samples from Alemana and Red Salud Clinics PWR in the city of Temuco. Aerosol samples were collected by using a large volume air sampler based on Coriolis technology (Coriolis µ, Bertin Technologies), and then total DNA was extracted using the commercial kit DNAeasy Power Soil Prokit (Qiagen). The quality and quantity of DNA was determined by Quantus™ fluorometer (Promega). Airborne bacterial composition was analyzed by amplification of hypervariable V3-V4 regions of the bacterial 16S rRNA and sequenced with an Illumina MiSeq Sequencer, (Illumina Inc.). Our preliminary results indicated that DNA contents was positively correlated with number of people in sampled PWR. At taxonomic level, a greater diversity of bacterial phyla was observed in PWR samples, including members belonging to Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes. This study represent one of the few approaches focused to study the microbial components present in PWR from medical centers in Chile, which are highly relevant for public health , particularly under pandemic emergency.

Closing conference

Advancing diagnosis for rare diseases through genomics.

Repetto Lisboa, Gabriela

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Rare Diseases (RD) are disorders with a prevalence of or less than 1/2000 individuals. There are more than 7000 known RDs, with a joint prevalence of 5%. The majority have a genetic cause and they can be chronic, complex, cause disability and early death. Exome and genome sequencing (ES and GS), coupled with detailed phenotypic evaluation and bioinformatic analysis have shown to be powerful tools for discovery of novel disease-causing genes and variants, and to reduce the "diagnostic odyssey" that many patients and families experience. Nevertheless, countries with limited genomic resources face challenges to implement RD programs, such as availability of clinical genetics and genomics specialists, sequencing and bioinformatics resources, and lack of health insurance coverage for genomic testing. With the aim of addressing these gaps and evaluating the effects of implementation of ES in diagnostic yield for persons with rare undiagnosed disorders, we developed an RD program with clinical evaluation and ES for individuals with congenital anomalies and/or intellectual disabilities with unknown etiology. To date, we have evaluated approximately 50 participants, and have reached a causative diagnosis in half of them, all with different conditions and genes involved. The majority harbored de novo variants. These results show the feasibility of developing RD programs in limited-resources settings, using combinations of local and outsourced capacities. With an implementation science approach, we are now evaluating the effects of reaching a diagnosis (or not), in health related quality of life, health care decisions, patient journey and cost-utility. We expect that the results will contribute to novel RD discoveries and guide clinical implementation of RD programs in the Chilean health care system.

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