BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

XX ANNUAL MEETING OF THE ARGENTINEAN BIOLOGY SOCIETY (SAB)

XVII MEETING OF THE URUGUAYAN SOCIETY OF BIOSCIENCES (SUB)

Second Biology Meeting of the River Plate

"NANOBIOTECHNOLOGY: SMALL SOLUTIONS FOR BIG PROBLEMS"

December 5th to December 7th, 2018

Buenos Aires, Argentina

The abstracts were evaluated by the Scientific Committee
of the Argentinean Biology Society

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

ARGENTINEAN SOCIETY OF BIOLOGY

EXECUTIVE BOARD AND SCIENTIFIC COMMITTEE

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

URUGUAYAN SOCIETY OF BIOSCIENCES

EXECUTIVE BOARD AND SCIENTIFIC COMMITTEE

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

MAIN LECTURES

A1
THE BIO-CHEMISTRY OF SILICA NANOPARTICLES: FUNDAMENTALS AND APPLICATIONS

Coradin T
Sorbonne Université, CNRS, Laboratoire de Chimie de la Matière Condensée de Paris, 75005 Paris, France.
E-mail: thibaud.coradin@sorbonne-universite.fr

Silica nanoparticles have become some of the most promising nanoscale carriers for drug delivery. However, very little is known about their interactions with living tissues *in vivo*. As a matter of fact, all gathered evidences point out that silica bio-chemistry runs along the same principles as silica chemistry in water, with a key role of dissolution-precipitation processes. In parallel no specific biomineralization or biodegradation pathways have been identified in animals so far while these are widespread in several photosynthetic organisms. Nevertheless, silica do have significant interactions with certain proteins and mammalian cells. Moreover, several studies have suggested that silica can be beneficial to health. Trying to put all these information together to get a whole picture remains difficult but allows to identify the next questions to be answered in this area, with important outcomes in medicine.

A2
GOLD NANOPARTICLES FOR BIOLOGICAL APPLICATIONS

Salvarezza R
Laboratorio de Nanoscopías y Fisicoquímica de Superficies (INIFTA-CONICET-UNLP).E-mail: robsalva@inifta.unlp.edu.ar

This presentation focuses on the synthesis, characterization and functionalization of gold nanoparticles (AuNPs) for applications in biology and medicine. These fascinating materials can be not only produced with controlled size and shape but also they can be post-functionalized to tune their surface properties and to immobilize different species able to interact with biological systems. In general small NPs (of about 2-4 nm core diameter) are obtained from Au^{3+} ions reduction by the addition of sodium borohydride in the presence of thiols. On the other hand, bigger NPs can be produced by Au^{3+} ions reduction using citric acid, sodium sulphide or thiosulphate. In the latter cases (sulphide and thiosulphate) near infrared resonant nanotriangles are obtained with potential application in thermal therapy of cancer. In all cases a careful characterization of the NPs is needed since the adsorbed species (thiols, citrate, sulphur species) resulting from the synthesis, which confer colloidal stability, may need to be replaced by other molecules in a post-functionalized step to improve biocompatibility, biorecognition, or for imaging or biosensing purposes. This exchange process requires a stronger affinity of the incoming molecules for the gold surface. For instance thiolated polyethylene glycol, frequently used to improve water solubility of NPs and biocompatibility, self-assemble on the NPs due to the strong affinity of the thiol group for the gold surface and the van der Waals forces acting between these large molecules. They also serve in many cases as anchor points for additional modifications of the NP's surface to confer therapeutic effects or diagnosis capability, and therefore have to be selected critically with regard to their intended application. However, the oxidation of the thiol S-head by oxygen yields weakly adsorbed molecules that are easily removed from the NP surface altering the engineered nanomaterial. Therefore, chemical stability is critical and must be included in the nanoparticle characterization. Also diffusion of gold adatoms can alter the size and shape of NPs by coalescence and Ostwald ripening changing their physical properties. Model systems using Au(111) and Au(001) single crystals, the most common faces present on the AuNP surfaces, are used to study and to predict the surface chemistry as the single crystals allow an easier characterization by surface analytical techniques and electrochemistry than the NPs. However, nanoparticles exhibit a large number of surface defects that can alter the stability of the adsorbed molecules and make the comparison not strictly valid. Finally, important issues such as those related to cell uptake, biodistribution and toxicological effects are still focus of present research.

A3
BIOMATERIALS AT THE NANOMAT CENTER IN URUGUAY

Pardo H[1], Mombrú AW[1]
[1]Centro NanoMat, Instituto Polo Tecnológico de Pando, Facultad de Química, Universidad de la República, Pando, Canelones, URUGUAY.E-mail: amombru@fq.edu.uy

The field of advanced materials research has been consistently evolving through the last decades, giving rise to new materials with improved properties that led to novel findings and applications for many purposes. This evolution and especially the innovative emphasis observed in the last years enhanced the interdisciplinary framework potential where many applications can arise. This potential and the advantage to study matter at a molecular level encouraged the search for applications, leading the development of this area with rates even higher than other fields of knowledge creation.
In this sense, the NanoMat Center of Facultad de Química, Universidad de la República, located at the Instituto Polo Tecnológico de Pando, has been working in several research lines, among which could be mentioned the following ones:

- Scaffolds for tissue replacement
- Biomaterials for biodegradable implantable drug delivery devices
- Micro and nano drug delivery systems

Thus, the presentation will show the work that has been performed in the fabrication of synthetic skin, ocular implants and the nanoencapsulation of anticarcinogenic drugs using different nanoparticle vehicles.

A4
MESOPOROUS MATERIALS AND THEIR BIOMEDICAL APPLICATIONS

Soler-Illia G J A A
Instituto de Nanosistemas, Universidad Nacional de San Martín, Av 25 de Mayo 1021, San Martín, BsAs, Argentina.
E-mail: gsoler-illia@unsam.edu.ar

In the last years, the nanotechnology community is aiming at producing complex materials that imitate the intricate multiscale architectures found in Nature. The combination of nanomaterials synthesis with self-assembly processes led to a significant advance in the production of hybrid inorganic-organic materials with hierarchical structures and localized functions. Our ability to produce highly precise complex architectures with well-defined localized functions opens the path to create matter that can change their physical or chemical properties in response to the environment, adapt to different media, or perform mechanical tasks upon sollicitation. Programmable nanosystems can be envisaged, in which confinement effects, responsivity, or collaborative functionality can be imparted into the structure through the control of positional chemistry of different chemical building blocks.In this presentation, we will illustrate the richness of this emergent field by focusing in mesoporous materials (MM), which can be highly controlled in terms of structure and composition control. These materials are made up by combining the self-assembly of inorganic nanobuilding blocks in the presence of supramolecular templates. The pore walls, surface or interior can ba modified at will with small molecular species, biomolecules, polymers or nanospecies. An amazing variety of chemical behaviors can be programmed into these structures, from tunable catalysis to light guiding or responsiveness to external stimuli.We will discuss examples of processed MM with projection to the biomedical community, as (bio)sensors, (bio)catalysts, separation devices, cell scaffolds and intelligent nanoparticles for the development of selective diagnostics and targeted delivery systems. A potentially infinite variety of nanosystems with externally controllable behavior is at our disposal, opening the path to design intelligent matter.

A5
THE ROLE OF NANOECOTOXICOLOGY IN THE DEVELOPMENT OF SUSTAINABLE NANOTECHNOLOGIES

Monserrat JM
Universidade Federal do Rio Grande-FURG, Instituto de Ciências Biológicas (ICB), Rio Grande, RS, Brasil.
E-mail: monserrat_jm@furg.br

Nanotechnology has brought impressive advances to many fields of modern science. Ecotoxicological data for aquatic organisms show a clear prevalence of studies employing freshwater organisms. However, and considering that agglomeration and deposition seems to be a common fate for several nanomaterials, data coming from estuarine and marine organisms are urgently needed, once ionic force agglomerates nanomaterials as fullerenes (C60). New strategies have been considered in recent years to cope with the increasing number of nanomaterials that must be evaluated about their potential toxicity. For example, implementation of *in silico* methods based in Docking Simulation (DS) appears to be an efficient alternative for the prediction nanomaterials hazard. Studies performed with mitochondria proteins (ADP/ATP carrier- ANT-1 and Voltage-Dependent Anion Channels- VDAC) showed they probably are targets for VDAC with single-walled carbon nanotubes (SWCNT).

A6
NANOSTRUCTURED MODULATORS OF BRAIN CELLS

Maysinger D
Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada.E-mail: dusica.maysinger@mcgill.ca

Biological and synthetic nanostructures can impact both glia and neurons. In the central nervous system (CNS), neurons represent only a small proportion (about 10%) of cells, whereas glial cells are the most predominant cell type. Non-targeted nanomedicines are mainly internalized by glia, in particular microglia, and to a lesser extent by astrocytes. Internalized nanomedicines by glia indirectly modify the functional status of neurons. The mechanisms of biochemical, morphological and functional changes of neural cells exposed to nanomedicines are still not well understood.
This presentation will provide a cross-section of morphological and biochemical changes in glial cells and neurons exposed to different classes of nanostructures (hard and soft). Specifically, at the neuronal level, examples will be provided to show how some nanomedicines alter the morphology of dendritic spines in the hippocampus. Effects of the pro-inflammogen lipopolysaccharide (LPS) and artificial dendritic structures on brain cells (neurons, microglia and astrocytes) will be shown and discussed in the light of neuroprotection and neural repair. Acknowledgments: Financial support for these studies was provided by CIHR and NSERC.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

YOUNG SCIENTISTS SYMPOSIUM

A7
MESOPOROUS AND HYBRID NANOSTRUCTURED BIOMATERIAL: THIN FILM COATINGS FOR ANTIMICROBIAL APPLICATIONS

Catalano PN
Departamento de Micro y Nanotecnología, Instituto de Nanociencia y Nanotecnología (CNEA-CONICET .
E-mail: catalano@cnea.gov.ar

The development of functional materials at the frontiers of cell biology, chemistry and materials sciences has been intensively explored in recent years. The possibility of characterizing, manipulating and organizing matter in the nanoscale, has great relevance in the development of film coatings that can be specifically designed to regulate cell behavior and to offer antibiofilm or antimicrobial properties. This could be achieved through the generation of mesoporous oxide thin films offering chemical and physical stability, transparency, high surface area, low cost, ease of production and the possibility to be tailored in order to control the pore size, shape and inter-connectivity. In particular, bacterial colonization of surfaces through the development biofilms can result in a powerful source of infectious diseases and/or promote corrosion of such surfaces. In this context, we developed mesoporous thin film coatings that prevent the formation of biofilm and that offer a high antibacterial effect. These coatings were based on oxide thin films with a defined nanotopography which significantly reduced the formation of biofilms with important implications in dental or orthopedic implants and prosthesis. We have also exploited the created thin film nano-reservoirs to adsorb silver ions obtaining a transparent ceramic coating that reduced the number of undesirable bacteria by more than 99.9% over long periods of time. These advantages resulted without modifying the texture, appearance or color of the coated surfaces and the commercial application reaches from domestic areas to health centers and other places where microbiological cleaning is critical, as food processing facilities, play areas or dining rooms. In another strategy, we used poloxamer block copolymers (Pluronic), typically templates in self-assembly techniques, to generate a hybrid nanostructured titania biomaterial coating with antibiotic-like activity. These nanopolymers are generally considered to be biologically inert and they were used to generate only bacteria repellent surfaces but keeps bacteria alive and as a latent threat. However, the inherent capabilities of these nanopolymers to kill bacteria have been largely overlooked. We found that nano-engineered Pluronic superstructures in fact possess an intrinsic broad-spectrum bactericidal activity similar to that shown for some antibiotics. This indicated that the appropriate control of superstructured mesophase architecture is key to bactericidal efficacy. Based on this finding, we developed a highly bactericidal coating against all tested Gram-positive and Gram-negative bacteria (>99.9 % kill) which moreover allows the adhesion and proliferation of mammalian cells. The inexpensiveness and ease of production of all here reported thin film coatings make them powerful tools for the development of a new generation of highly effective antimicrobial nanomaterials.

A8
MAGNETIC NANOSYSTEMS AS THERANOSTICS AGENTS: A PERSPECTIVE FROM THE DESIGN TO THEIR POTENTIAL APPLICATIONS IN THE TREATMENT AND DIAGNOSTIC OF HIGH IMPACT DISEASES

Azcona P, Martin J, Montiel Schneider G, Agotegaray M and Lassalle V
Instituto de Química del Sur, Departamento de Química, (UNS-CONICET), Bahía Blanca, Pcia. Bs. As.
E-mail: veronica.lassalle@uns.edu.ar

Nanotheranostics aims to combine imaging diagnostic and therapy in a single nanotechnology vector to the detection and treatment of critical diseases. Due to the high intra and inter-variability found in tumors, nanotheranostics, have emerged as indispensable tools for personalized therapy. The ability to engineer nanomaterials to interact with tumor cells at the molecular level can significantly improve the effectiveness and selectivity of therapy to cancers that are currently difficult to treat. By multiple reasons, magnetic nanoparticles (MNPs) appear as attractive components for designing theranostics. One of the most interesting properties is their ability as MRI contrast agent to produce predominant T_2 relaxation effects with excellent sensitivity without the risks associated to the use of gadolinium based agents. On the other hand, MNPs may promote active target by means of an external magnetic field, increasing the efficiency of the therapy by using lower drug doses and avoiding secondary effects. The goal of this research is to design efficient magnetic nanotheranostics to implement in the early diagnostic and therapy of oncological diseases. To this end, iron oxide MNPs have been employed as raw materials and diverse modifications have been implemented from the synthetic pathways to achieve nanovectors with ability to: i- Passive targeting, (ii) active targeting and (iii) stimuli-mediated targeting. The first one is achieved by simply exploiting the vasculature properties of the tumor that induce the MNPs accumulation by EPR effect. Active targeting is achieved by conveniently modifying the MNPs surface including specific ligands able to preferentially bind to biological markers overexpressed in tumors. iii. The stimuli targeting is achieved by means of an external magnet, allowing the nanovector reach the tumor site. The developed work includes the coating of MNPs with folic acid (FA), mannose (MAN), and silica. FA and MAN were selected due to their affinity to interact with folate and mannose receptors, respectively, that are overexpressed in colon rectal and other cancerous diseases. Silica was chosen because its bioactive properties regarding bone system allowing the treatment of bone

diseases and favouring the bone metabolism. The loading of drugs related to the selected pathology provide the therapeutic action. In this regard, MNPs modified with FA have been loaded with doxorubicin and MNPs coated MAN with morine, a natural flavonoide with antitumoral activity. Raloxifen and diclofenac have been the selected drugs to load on silica-coated MNPs. The performance of the designed nanosystems as contrast agents has been evaluated in a clinical MRI equipment to verify their integral capabilities as nanotheranostics. *In vitro* assays have been developed aiming to examine their cytocompatibility, uptake, and therapeutic effects employing colon cancer and healthy endothelial cells. Biocompatibility, biodistribution, blood compatibility and magnetic targeted assays have been developed using different *in vivo* models. The knowledge achieved in terms of therapeutic and diagnostic capabilities of these kinds of theranostics appears as highly promising in the context of the personalized therapy, especially concerning to oncological diseases.

A9
DESIGN, SYNTHESIS AND BIOMEDICAL APPLICATIONS OF MULTIFUNCTIONAL SILICA PARTICLES

Desimone MF
Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina.
E-mail: desimone@ffyb.uba.ar

The use of silica nanoparticles in biomedicine is discussed in terms of the methods of silica nanoparticle production and grafting. Moreover, the physicochemical properties, drug delivery and uses in nanocomposite approaches is presented. Initially, the effect of silica particle surface functionalization on antibiotic sorption was studied, enlightening the role of electrostatic and hydrophobic interactions. Moreover, core–shell silica particles allowing for the dual delivery of gentamicin and rifamycin were prepared. Secondly, silica–collagen type I nanocomposite hydrogels are evaluated as medicated dressings to prevent infection. Indeed, collagen-silica nanocomposites allowing for the prolonged release of two topical antibiotics are promising medicated dressings to prevent infection in wounds. Finally, the analysis of the effect of these nanoparticles with monocytes and red blood cells highlights the complex interactions involved in these inherently sophisticated nanomaterials.

A10
"CLICK-MAGNETIC NANOPARTICLES": A HIGHLY VERSATILE SUPPORT FOR APPLICATIONS IN DIFFERENT AREAS

Mendoza C
Departamento Estrella Campos, Área Química Inorgánica, Facultad de Química, Udelar.
E-mail: cmendoza@fq.edu.uy

In recent years magnetic nanoparticles (MNPs) are attracting an increasing attention in many different fields due to their intrinsic properties such as high surface area, low toxicity and superparamagnetic behavior. This latter property, in particular, allows nanoparticle motion control by application of an external magnetic field, enabling the easy separation and recovery of the MNPs from the reaction medium. Metal oxide nanoparticle of the ferrite type present an outer surface rich in hydroxyl groups that can be readily functionalized by grafting with a variety of □-substituted 1,1,1-trialkoxysilanes. In particular 3-azidopropyltriethoxysilane can be grafted onto magnetite nanoparticles affording azide functionalized nanoparticles ("click-magnetic nanoparticles") that can be further modified by copper (I) catalyzed 1,3-dipolar cycloaddition of an azide and an alkyne (CuAAC, the most popular version of *click chemistry*). This strategy has been widely employed in the functionalization of a variety of materials (polymers, polysaccharides, silica surfaces and nanoparticles) for its valuable characteristics such mild reaction conditions, high reaction yields and no by products. We have employed *click chemistry* as a convenient strategy for the anchoring of a wide range of structures (metal coordinating macrocycles, organocatalysts and β-cyclodextrin onto Fe_3O_4 nanoparticles. The macrocycle functionalized MNP were used in the extraction of heavy metals (for example Pb^{2+}) from aqueous solutions. The organocatalysts functionalized MNP were investigated as catalysts for the asymmetric aldol reaction in aqueous and organic media and the asymmetric Michael addition of propanal to nitroolefins. Finally a supramolecular approach has been followed to support adamantyl substituted proline organocatalysts onto the surface of magnetite nanoparticles decorated with a β-cyclodextrin motif. The resulting magnetic nanoparticles were used as modular, magnetically recyclable catalysts in the asymmetric aldol reaction in water with the possibility of easily dismantling and re-complexing with another suitably catalytic unit (replaceable functional cargo). All these examples show the high versatility of "click-magnetic nanoparticles" as anchorage point to built functional MNP for different applications.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

SYMPOSIUM OF BIOLOGY SOCIETIES

A11
FORMULATION, QUALITY CONTROL AND SAFETY ISSUES OF NANOTECHNOLOGICAL MEDICINAL PRODUCTS

Bianco I, Casado C, Dabbene V, Rizzi C, Ardanaz E, Margaría F, Mizutamari K.
CEPROCOR – CONICET - Universidad Nacional de La Rioja.

Although we have seen great advances, most human pathologies remain incurable because many patients either do not respond or relapse to treatment. Several lines of research are disclosing new therapeutic targets which lead to the development of new active drugs. However, there are still unsolved problems related to stabilization of the pharmaceutical ingredient in aqueous and biological media, pharmacokinetic and pharmacodynamic profiles and cellular uptake to name just a few. In this context, nanotechnology with the emerging tools of nanoengineering offers many possibilities to guide the design of new products with improved safety and efficacy. The presence of several reacting groups and the sensitivity of their properties to small changes in composition make nanocarriers tunable not only to modify their stability in a particular environment but also to respond to changes in biological situations in the right place and time frame.
This talk will summarize the main preparation methods and formulation strategies of nano and microcarriers designed for drug delivery applications and will attempt to give a glimpse on how their structure, shape, physico-chemical properties and chemical composition may affect their overall stability and interactions with biological systems. A challenge with nanocarriers is that their biological activities depend not only on their chemical composition but also on those of the structures formed by them. From this, a very important issue that emerges is that nanocarriers frequently display an intrinsic bioactivity (i.e.: immunomodulatory). Therefore, it should be stressed that nanocarriers cannot be considered as inert, biocompatible excipients. As an approach to the rational design of new pharmaceutical products nanoengineering is providing new tools for the precise control of the properties of nanocarriers.

A12
THE PROTEIN AsES ACTIVATES THE INNATE IMMUNITY IN STRAWBERRY

Díaz Ricci JC, Perato M, Hael-Conrad V, Martos GG.
Instituto Superior de Investigaciones Biológicas (INSIBIO), Universidad Nacional de Tucumán - CONICET, Tucumán, Argentina.
E-mail: juan@fbqf.unt.edu.ar

We have previously reported that strawberry plants (*Fragaria ananassa*) treated with the defense elicitor AsES exhibited a higher resistance against the fungal pathogens *Colletotrichum actutatum* and *Botrytis cinerea*. It was hypothesized that the effect was due to the activation of the plant innate immunity. With the aim to test that hypothesis we have investigated which defense signaling pathway was activated in plants treated with AsES. Strawberry leaves of the cv Pájaro were treated w ith AsES and harvested at different times to evaluate the level of expression of different genes associated to the salicylic acid (SA) and ethylene (ET) signaling pathways. Plants treated with water were used as control. The evaluated genes associated to the SA pathway were: *PR1, ISC1, PAL1, RBOHD-F, CHI1.2, SAMT, SAMES* and *SAGT*; and the genes associated to the ET pathway were: *ETR1, ACS, ACO, CTR1, ERS1, EIN2, PDF1.2*. Other genes associated to defense response such as: *YB30 (*HR marker); *FLS* (flavonol synthesis)*; GSL5* (callose synthesis); *PRX27* (lignin synthesis), and *PG* (cell wall degradation) were also investigated. The accumulation of SA and ET production were also analyzed in plants at different times after AsES treatment. Results obtained revealed that AsES activates the innate immunity by using, in an early stage, a mechanism independent to *RBOHD* and *RBOHF*, that is an oxidative burst independent to NADPH oxidase, and activates both signaling pathways (i.e. SA and ET), but in a sequential mode, revealing a cross-talk between them.

A13
DESIGN OF NANOSTRUCTURES AS DRUG CARRIERS AND NANO-INGREDIENTS FOR FUNCTIONAL FOODS

Kassuha DE
Instituto de Investigaciones en Ciencias Químicas. Facultad de Ciencias Químicas y Tecnológicas. Universidad Católica de Cuyo. San Juan, Argentina.E-mail: dkassuha@uccuyo.edu.ar

While pharmaceutical drugs have revolutionized human life, there are several features that limit their full potential including low solubility, poor bioavailability, high drug dose, undesired side effects and resistance to the treatment. An emerging technology that is geared towards improving overall therapeutic efficiency resides in drug delivery systems including the use of polymeric nanoparticles (NP) which have found widespread use in therapeutics. These polymeric NP can provide targeted drug delivery, increase the circulation time in the body, reduce the therapeutic indices with minimal side effects, and accumulate in cells without activating the mononuclear phagocyte system. Given the inroads made in the field of nanodelivery systems for pharmaceutical applications, our work emphasizes the importance of Polymeric nanocarrier system for drug delivery in chemotherapy and hypertension treatment.

The work aims to develop, to characterize and to evaluate *in vitro* and *in vivo* biological activity of polymeric NP obtained by electrospraying /electrospinning thechniques. For pulmonary hypertension, we developed bosentan/ε-polycaprolactone NP (BOS-NP) and for arterial hypertension, we developed anandamide/ε-polycaprolactone NP (AEA-NP) which were characterized by means of DSC, TGA, SEM, FTIR and XRPD. For BOS-NP, the drug loading was 25.98% and the encapsulation efficiency was 58.51%. Additionally, the dialysis membrane method during 24 and 72 hours showed prolonged and controlled release of BOS, in comparison to non-encapsulated BOS. The release data showed to fit with the Cubic Root kinetic dissolution. For AEA-NP, we also characterized the NP and demonstrated that they can induce the expression of iNOS and NO levels and to decrese Na^{+1}/K^{+1} ATPase activity in HK2 cells in a dose dependent manner. We also are working in the development of functionalized NP systems with more than one cytotoxic drug with specific aptamers to lung cancer cells can allow its release in desired that cells and avoid his arrival to another organs or tissues, improving efficiency of the treatment and reducing drug resistance and side effects. Additionally we developed nano-ingredients from *Brasicca* sp plants from Cuyo region for food bio-fortification and cancer chemio-prevention.

A14
MICRO/NANOPARTICLES OBTENTION DUE TO THERMODYNAMIC INCOMPATIBILITY BETWEEN FOOD GRADE BIOPOLYMERS

Nielsen NS[1], Bea L[1], Veyñ M[1], Ingrassia R[1,2,3], Risso P[1,2,3], Hidalgo ME[1,3]
[1]Facultad de Ciencias Bioquímicas y Farmacéuticas-Universidad Nacional de Rosario (UNR); [2]Facultad de Ciencias Veterinarias-UNR; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). E-mail: mhidalgo@fbioyf.unr.edu.ar

Foods contain proteins (PT) and polysaccharides (PS) in form of complex multicomponent mixtures, and so it is often difficult to disentangle the separate roles of the biopolymers in terms of their functional properties, responsible for the structure, mechanical, textural, and other physicochemical properties. These functional properties are affected by the interaction between biopolymers and other components of food systems. Therefore, the study of the interactions in PT-PS mixtures and their adequate formulation may contribute to the elaboration of food products with special microstructure, rheological, and textural characteristics. Segregative phase separation by thermodynamic incompatibility is commonly observed in ionic and nonionic biopolymer mixtures. At low biopolymer concentrations, the mixture remains a single phase. However, above a critical concentration ratio, PT-PS mixtures form a system with two separated phases, in which each phase is enriched in one of the biopolymers. The limited thermodynamic compatibility between PT and PS may be exploited to obtain micro/nanoparticles, by controlling the size of the aggregates (e.g. by selecting the PS, the temperature, and the relative concentrations of PT and PS). On the other hand, a process that has gained the attention of the food industry is the acidification of milk by addition of glucono-□-lactone (GDL). It is known that the gelation process rate affects the hardness and elasticity of the resulting acid gels. In PT-PS mixtures, the presence of PS may produce changes in the protein gel formation kinetics, leading to the formation of gels with different microstructural and textural characteristics. This behavior may be due to the competition between the gelation process and the phase micro-separation generated by thermodynamic incompatibility. In this work, PT and PS assayed were bovine sodium caseinate (NaCAS) and soy protein isolate (SPI), and locust bean gum (LBG) and tara gum (TG), respectively. For each PT-PS mixture, thermodynamic incompatibility was analyzed. Besides, the effect of the presence of PS on the PT acid gels formation, the textural and the microstructure characteristics of acid gels were studied. In general, the presence of PS affected the formation and the microstructure of protein acid gels and, at a certain ratio of concentrations, protein microparticles were obtained. These microparticles may be formed due to a competition between the kinetics of phase separation and the acid gelation process. Under these conditions, these acid gels present different microstructural and textural characteristics.

EXPERT SCIENTISTS SYMPOSIUM

A15
RELEVANT TOXICOLOGICAL ASPECTS FOR THE DEVELOPMENT OF SAFE NANOMATERIALS

Evelson P
Universidad de Buenos Aires Facultad de Farmacia y Bioquímica., Cátedra de Química General e Inorgánica. Instituto de Bioquímica y Medicina Molecular (IBIMOL -UBA-CONICET). E-mail:pevelson@gmail.com

Current advances in nanotechnology have led to the development of different nanomaterials and nanodevices for several applications, such as semiconductors, microelectronics, cosmetics, catalysts, and drug carriers. However, there is an increasing concern surrounding their potential harmful effects on human health. Therefore, it is important to establish the toxicological mechanisms triggered by the exposure to nanomaterials, since it would provide valuable information about the possible hazards and risks elicited by each one of them. Metallic nanoparticles (NP) and metal-coated NP are among the most widely used materials in nanotechnological applications. The respiratory system has been pointed out as one of the main portal of entry for the exposure to NP, together with the gastrointestinal tract and the skin. Due to their small size, NP can easily reach the lower respiratory tract after inhalation. Moreover, it has been suggested that lung injury after the exposure to NP could trigger a local response with adverse health effects over distant

organs such as the heart and brain. The occurrence of oxidative stress and an inflammatory response have been postulated to play a central role in the harmful health effects exerted by NP exposure, where transition metals, such as Ni, Fe and Ag, play a central role, due to their ability to participate in Fenton-like chemical reactions and induce the production of reactive oxygen species production. A better understanding of the mechanisms underlying NP induced adverse health effects would allow a more targeted approach to face the toxic effects of NP, and could possibly provide different ways to develop safer nanomaterials.

A16
APPLICATIONS OF MAGNETIC NANOPARTICLES IN MEDICINE: HYPERTHERMIA AND PEROXIDASE CATALYTIC ACTIVITY

Lima Junior E, Moreno Maldonado AC, Winkler EL, Mojica Psciotti ML, Raineri Andersen M, De Biasi E, Vasquez Mansilla M, Troiani H, Zysler RD
Instituto de Nanociencia y Nanotecnología (INN). Nodo Bariloche. CNEA/CONICET, Argentina.Laboratorio Resonancias Magnéticas, Centro Atómico Bariloche, S.C. de Bariloche, RN, Argentina.E-mail: zysler@cab.cnea.gov.ar

The use of magnetic nanoparticles has been proposed since the late 1990s. Among them are different methods of recognition and treatment of diseases. These applications are based on the fact that nanoparticles have a high surface/volume ratio, and functionalization of them has a high efficiency in terms of the activity that is intended with them (recognition, protein expression/antibodies, separation of molecules or action of medicines). On the other hand, the superparamagnetic properties of these nanoparticles promote a strong interaction with an applied magnetic field. In the case of DC magnetic fields, the attractive force is used in the concept of *drug delivery*; and with alternating magnetic fields, the heating of the particles is promoted, which it is utilizable in the treatment of magnetic fluid hyperthermia. In recent times, as the nanoparticles can be multifunctional, the idea to combine different properties of these nanostructures has been generalized. As an example of this, the idea of theranostics that combines diagnosis and treatment in the same material has been developed. The iron oxide nanoparticles (IONP), which have a wide range of use in medical applications (drug delivery, contrast agent, molecule separation, magnetic fluid hyperthermia, etc.), have the ability to catalyze this reaction, and therefore be facilitators of cell death. Fenton's reaction is known since the late nineteenth century. This is an oxidative reaction where Fe^{+2} ions catalyze a peroxidase-like reaction with the production of free radicals (Reactive Oxygen Species - ROS). These radicals can cause cell death. This fact can be use in cancer treatments combined with the hyperthermia treatment. In this presentation will be shown results of magnetically heating of IONP for use in hyperthermia treatment, and the catalytic activity of IONP and the quantification of free radicals produced by means of electronic paramagnetic resonance spectroscopy (EPR).

A17
NANOTECHNOLOGY AS A TOOL FOR THE TREATMENT OF ENDOCRINE RESISTANT BREAST CANCER

Diaz Bessone MI, Simian M
Laboratorio de NanoBiología, Instituto de Nanosistemas, Universidad Nacional de San Martín.E-mail: msimian@unsam.edu.ar

Seventy five percent of breast tumors express estrogen receptors (ERs). Tamoxifen, a selective ER modulator, is the most widely used therapy for these patients. However, about one third of treated patients eventually develop tamoxifen resistance and cancer reappears. We previously showed that fibronectin, when bound to cell surface β1-integrins, induces tamoxifen resistance in breast cancer cells. Additionally, we recently demonstrated that β1-integrin co-localizes with ERα at the cell membrane and in endosomes in breast cancer cell lines and in human normal and neoplastic tissue samples. In the presence of FN, β1 prolongs ERα's half-life and strengthens its transcriptional activity. We hypothesized that targeting of β1 integrin/FN interaction in combination with ER blockade could be an effective strategy to avoid emergence of FN-induced Tam resistance and the selection of breast cancer stem cells - events associated with cancer recurrence. Nanoparticles (NPs) provide new features and functions that are different from those present in the individual components. In particular, small size, high surface area/volume ratio and multifunctionality, make NPs attractive as carriers for drugs and diagnostics. The aim of this project was to investigate the effectiveness of multifunctional tamoxifen –loaded NPs, compared to free tamoxifen, for the treatment of breast cancer. To do so we to designed iRGD-coated and tamoxifen –loaded polimersomes and tested their efficacy on MCF-7 breast cancer cells in vitro and in vivo. Our results show that at equivalent concentrations, tamoxifen had a greater effect on cell viability when carried in NPs. iRGD-coated tamoxifen NPs reduced stem cell growth, contrary to what is observed with free tamoxifen, and inhibited ER's transcriptional activity, as expected. Cell uptake of NPs is increased when they are coated with iRGD, as well as in vivo tumor homing. Our results suggest that iRGD-coated tamoxifen NPs could be a rationale alterative for the treatment of ER positive breast cancer, leading to increased disease-free survival.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A18
QUALITY CONTROL IN THE SYNTHESIS, CHARACTERIZATION, AND BIOCONJUGATION OF GOLD NANOPARTICLES

Méndez E[1], Fagúndez P[1,2], Tosar JP[2], Botasini S
[11]Laboratorio de Biomateriales, Instituto de Química Biológica, Facultad de Ciencias, Universidad de la República (Uruguay) y [2]Unidad de Bioquímica Analítica, Centro de Investigaciones Nucleares, Facultad de Ciencias, Universidad de la República (Uruguay).E-mail: emendez@fcien.edu.uy

Despite the wide use of gold nanoparticles in drug delivery and bioanalytical protocols, the urgency for the development of new assays left aside the quality control of the whole procedure of synthesis, characterization, and bioconjugation. Every step in the procedure should be accompanied by a proper check of its effective execution. Based on this fact, in the last years, we were working in the development of methods that qualify the procedure of bioconjugation of gold nanoparticles (AuNPs) taking as the initial step the synthesis of gold nanoparticles itself. Not only the qualitative changes of every step are assessed, but also metrological data are considered.The main techniques considered are UV-Vis absorption spectroscopy (UV-Vis), Fourier-transformed infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), Z-potential measurements (Z potential), Electrophoretic mobility (El-mob), and colloidal stability (CS). These techniques were applied to the whole procedure for the synthesis of bioconjugated gold nanoparticles. The metrological aspects of AuNPs remain as a challenge. The absence of data on the extinction coefficients for different capping agents modified gold nanoparticles (only extensive data tables for citrate-capped AuNPs exist) makes the quantification of the resulting AuNPs difficult. The aid of TEM results to calculate such concentration suffers from the lack of perfect sphericity of the nanoparticles, which is also a key assumption in DLS measurements. Finally, the purification step should be carefully controlled in order to avoid capping agent stripping from the AuNPs surface, and induced aggregation.The modification step with self-assembled monolayers is a well-known procedure, but lacks of a reliable technique that confirms that the procedure took place. FTIR, UV-Vis, and DLS do not provide conclusive data. The bioconjugation step based on carbodiimide chemistry left the biorecognition event as the single quality control of the target product. However, proper control of the purity in the assay is needed to achieve such a goal. Electrophoretic mobility and Z-potential measurements help in the confirmation of the synthetic process at this stage. The information provided by each technique in relation to the protocol step will be discussed. The metrological basis of UV-Vis and TEM will be presented, with the adequate formulation of concentration units. A novel technique based on the assessment of the colloidal stability developed in our laboratory provides simple and automated quality control in most of the steps.

SHORT COMMUNICATIONS

ENDOCRINOLOGY AND METABOLISM

A19
EFFECTS OF A CONTINUOUS AND PROLONGED IMT504 TREATMENTIN DIABETIC FEMALE NOD MICE

Bianchi S[1], LavignolleHeguy MR[1], Montaner A[2], Libertun C[1,3], Lux-Lantos V[1] and Bianchi MS[1].
[1]IBYME-CONICET, [2]ICT Milstein-CONICET y [3]Facultad de Medicina, Univ. de Buenos Aires; Bs As, Argentina.
E-mail: msbianchi@ibyme.conicet.gov.ar

We have shown that the immunomodulatory oligonucleotide IMT504 (IMT) promotes marked recovery of toxic diabetes in rats that correlates with early expression of progenitor cell markers but without altering immune parameters. IMT also improves immunodependent diabetesin mice, diminishing glycemia and reducing leukocyte islet infiltration. Besides, a short-term IMT treatment induces an early recovery of glucose homeostasis and insulitis in NOD mice,a model of type I diabetes. Here, we evaluated the effects of a continuous chronic IMT treatmentin NOD diabetic mice, more similar to what could eventually be used in humans.Diabetic female NOD mice (non-fasted glycemia (Gly) levels between 250-350 mg/dl were s.c. implanted with constant drug release pumps (Alzet osmotic pumps) with a capacity for 28 days, loaded with IMT (total dose/ per day: 20 mg/kg body weight (BW) or saline (diabetic control: DC) (day1). Gly and BW were determined once a week during the treatment. The weekly food intake was determined. On day 21, glucose tolerance (ipGTT) and insulin secretion (IST) testswere performed. On day 28, fasted mice were sacrificed, blood samples and pancreases collected for hormonal determinations and histological studies respectively. We observed that 22% of NOD mice showed spontaneous reversion of the diabetic condition whereas IMT treatment ameliorated Gly in 78% of mice (X2: $p<0.05$). IMT-treated animals quickly attained normal Gly, which was maintained until the end of the experiment [ANOVA, interaction $p<0.001$; Gly (mg/dl) day 1= DC: 318±10, IMT: 301±12 vs day 28: DC: 540±23, IMT: 151±17; $p<0.01$]. IMT induced a significant recovery on glucose clearence [GTT: ANOVA: interaction $p<0.02$, 0 min= DC: 390±62 vs IMT: 141±23, $p<0.002$; 30 min= DC: 581±56 vs IMT: 239±32, $p<0.004$; 120 min DC: 422±53 vs IMT: 149±23, $p<0.001$] as well as on insulin secretion in response to the glucose overload [IST: ANOVA: interaction $p<0.02$; 0 min= DC: 0.16±0.14 vs IMT: 0.22±0.03, ns; 10 min= DC:

0.15±0.01 vs IMT: 0.33±0.05, p<0.04; 60 min DC: 0.15±0.02 vs IMT: 0.18±0.02, ns]. Body weights did not differ between groups. IMT treatment prevented food intake increase observed in DC group [ANOVA, interaction p<0.001; week 1= DC: 29.8±1.3 vs IMT: 26.0±1.4, ns; week 4= DC: 58.1±3.4 vs IMT: 29.0±1.3, p<0.001]. Besides, beta cell function was improved in IMT-treated animals [HOMA beta cell= DC: 12.3±6.3 vs IMT: 125±48.3, p<0.001]. These results demonstrate the effectiveness of prolonged and constant IMT treatment in promoting a significant improvement in terms of glycemic control and the diabetic condition in this spontaneous autoimmune diabetes model. (CONICET, UBA, ANPCYT, Fund. Williams, Fund. René Barón).

A20
NOTCH PATHWAY MODULATES VEGF AND ENDOCAN-MEDIATED ANGIOGENESIS IN THE BOVINE MAMMARY GLAND DURING PUBERTAL DEVELOPMENT

Bonadeo N[1], Chimento A[1], Perrone S[1], Becú-Villalobos D[2], Cristina C[1], Lacau IM[2]
1-CIBA-CITNOBA UNNOBA-CONICET, Newbery 261, Junín, province of Bs. As.; 2- IBYME-CONICET, Obligado 2490, CABA.
E-mail: nadia_bonadeo@hotmail.com

We have previously described a differential expression and activation of Notch receptors during bovine mammary gland development. In this work, we aimed to study the relation between Notch pathway components, proliferation and angiogenesis during pubertal development. In Holstein heifers of 20, 30, 40 and 70 weeks of age, we studied αSMA and PCNA expression by immunohistochemistry and we observed an increased microvascular density at 30 weeks of age (p<0.1). By Western blot studies, we also found an increase in the endothelial cells marker CD34 at 30 weeks of age and a decrease at 40 weeks of age ($p=9.8x10^{-10}$). Furthermore we measure Hes1, Hey1 and Hey2 mRNA, target genes of Notch pathway, and we found an increase of Hey1 gene expression at 40 weeks of age ($p=1x10^{-6}$) and an increase of Hey2 at 70 weeks of age (p=0.0002). Moreover Real time PCR studies showed that the expression of the Jagged1 ligand also increased at 30 weeks of age (p=0.007) and Delta1 ligand at 70 weeks of age (p=0.044). We also determined the mRNA levels of the angiogenic markers VEGF and Endocan. We observed an increase in Endocan at 40 weeks of age (p=0.016) and an increase of VEGF at 70 weeks of age (p=0.0078). We found a positive correlation between the expression of Notch1 receptor and Hey1 (p=0.0017; r=0.67) and Hey2 (p=0.0041; r=0.67), and between Notch3 receptor and Hey1 (p=0.017; r=0.56). Likewise, both target genes correlate positively with VEGF (p=0.0001; r=0.75) (p=0.024; r=0.53) and Endocan (p=0.0024; r=0.59) (p=0.016; r=0.53). In conclusion, we observed Notch pathway activation at all studied ages, with significant increments at the time of puberty onset (30 and 40 weeks of age). We also observed more angiogenesis at this peripubertal stage. Our results support the Notch pathway activation during bovine mammary gland development through Hey1 and Hey2 target genes and would indicate a role of Notch pathway in the developmental angiogenesis mediated by VEGF and Endocan.

A21
IN SEARCH OF THE MECHANISM OF ACTION OF IMT504 IN BETA CELLS (MIN6B1)

Converti A[1], Bianchi MS[1], Montaner A[2], Lux-Lantos V[1], Libertun C[1,3], Bonaventura MM[1,4].
[1]Instituto de Biología y Medicina Experimental (IBYME), [2]Instituto Milstein-Fundación Cassará, [3]Universidad de Buenos Aires, [4]Universidad de Gral San Martín. E-mail: mmbonaventura@gmail.com

We have previously demonstrated that treatment with IMT504 promotes significant improvement in the diabetic condition in diverse animal models. We have also shown effects on gene expression in freshly isolated islets from diabetic, *in vivo* IMT504-treated animals and in *in vitro* IMT504-treated beta cells (MIN6B1). Based on these results, here we started to investigate the possible mechanism of action of IMT504 on beta cells. Other authors have previously demonstrated that IMT504 modifies $GSK3_\beta$ and AKT phosphorylation in other cell types. Since $GSK3_\beta$ and AKT participate in the regulation of beta cell function, we started to evaluate this signaling pathway in MIN6B1 cells.
MIN6B1 cells were cultured in DMEM with 20 mM glucose, 15% SFB, 71 µM β-mercaptoethanol. Phosphorylation of proteins of interest was analyzed by Western Blot. To study enzyme activation, cells were stimulated for 0, 5, 15, 30 and 60 min with 4 µg/µl of IMT504 (IMT4) in DMEM, 20 mM glucose, 0,5% BSA, 71 µM β-mercaptoethanol (n=6). For more prolonged stimulation, cells were incubated for 24 or 48 hs with 0 (C), 2 (IMT2), 4 (IMT4) and 8 µg/µl (IMT8) of IMT504 in the same medium (n=3-4). At each time point cells were lysed in sample buffer for Western Blot analysis. $pGSK3_\beta/GSK3_\beta$ ratio (A.U.) and pAkt/Akt ratio (A.U.) were calculated and informed as fold increase with regard to 0. At 60 min, $pGSK3_\beta/GSK3_\beta$ ratio was increased compared to 0 min (ANOVA with repeated measures: $pGSK3_\beta/GSK3_\beta$ ratio (A.U.) 0 min = 1, 5 min = 1.15 ± 0.06, 15 min = 1.20 ± 0.05; 30 min = 1.54 ± 0.14; 60 min = 2.04 ± 0.17, 60 min different from 0 min, p<0.03). No significant differences were observed in pAkt/Akt ratio (ANOVA with repeated measures, NS). For 24 and 48 hs we found no significant differences between groups.
Our results, although preliminary, suggest that IMT504 could exert its actions on beta cells through a pathway that includes $GSK3_\beta$ phosphorylation. Further studies must be done to elucidate the implications of $GSK3_\beta$ phosphorylation on beta cell recovery in diabetic animals.FUNDING: CONICET, UBA, ANPCYT, FUNDACIÓN WILLIAMS, FUNDACIÓN RENÉ BARON.

A22

IN-VITRO EXPOSURE TO BISPHENOL A AND BENZOPHENONES 2 AND 3 ALTER GNRH GENE EXPRESSION IN MATURE GNRH NEURONS

Marigliano C[1], Libertun C[1,2], Lux-Lantos V[1], Fernandez M[1].
1-Laboratorio de Neuroendocrinología-Instituto de Biología y Medicina Experimental (IByME-CONICET) and 2-Facultad de Medicina-UBA.E-mail: mfernandez@dna.uba.ar

Bisphenol A (BPA), a monomer of polycarbonate plastics, and Benzophenones (BPs), UV-filters, are endocrine disrupting chemicals (EDC). Previously we showed that twenty-four-hour pre-treatment with BPA and BP2 ($1x10^{-9}$M) inhibit Kisspeptin (Kiss)-induced GnRH gene expression in immature GnRH neurons, GN11 cells, whereas BP3 did not have this effect. In this study we investigated the in-vitro effects of BPA, BP2 and BP3 on GnRH gene expression in mature GnRH neurons; we used the GT1-7 cell line (Dr Pamela Mellon, UCSD, USA) and isolated hypothalami from adult Balb/c males. Hypothalami were incubated in Krebs-Ringer medium in the presence or absence of BPA, BP2 or BP3 ($1x10^{-9}$M) or medium alone (C) for six hours. After the incubation, hypothalami were homogenized in Tri-Reagent (Molecular Research Center, OH, USA) and mRNA extracted for *Gnrh* analysis by qPCR. Media were removed and kept -20 ºC for GnRH determination (RIA). GT1-7 cells were cultured in 12-well plates (200000 cells/well) in DMEM supplemented with 10% FBS, pyruvate and Penicillin/Streptomicin (complete medium). After 24 hours, media were changed to DMEM without phenol red, supplemented with 10% charcolized-FBS, pyruvate and Penicillin/Streptomicin (stimulation medium). Cells were incubated with BPA, BP2, BP3 ($1x10^{-9}$M) or vehicle (C) in stimulation media for 24 hours, and after this, media were changed, stimuli renewed and cells further stimulated with Kiss1 ($1x10^{-9}$M) for 4 h to analyze gene expression. RNA was extracted using Tri-Reagent and *Gnrh* analyzed by qPCR. RNA (1ug) was reverse transcribed and Real-Time PCR performed using specific primers. Cyclophilin B (*Ppib*) was used as housekeeping gene and results were analyzed using the mathematical model of Pfaffl et al (*Nucleic Acids Res* 29: e45, 2001). Hypothalami exposed to BPA showed increased GnRH expression compared to C (Relative expression: C=4.6±0.9; BPA=10.7±2.2; ANOVA: BPA vs C $p<0.05$, $n=9$), whereas neither BP2 nor BP3 had any effect (ANOVA: ns). GT1-7 cells exposed to C showed Kiss induced GnRH gene expression, while the exposure to the EDC abolished this response (Relative gene expression: C-Basal=1.0±0.1, C-Kiss=1.6±0.3, BPA-Basal=1.3±0.2, BPA-Kiss=1.1±0.2, BP2-Basal=1.2±0.1, BP2-Kiss=1.4±0.2, BP3-Basal=1.3±0.3, BP3-Kiss=1.1±0.2; Repeated measures Two-way ANOVA: Interaction $p<0.05$, $n=6$). Our results show that the exposure to EDC alters the physiology of mature GnRH neurons. Further studies are needed to dissect the mechanisms involved in these effects. Funding: CONICET, ANPCyT, UBA, International Society for Neurochemistry, Asoc. ORT Arg., Fund. R. Barón, Fund. Williams.

A23

CURCUMIN AND HEME OXYGENASE-1 EFFECTS ON LEYDIG CELLS STEROIDOGENESIS AND PROLIFERATION

Raices T[1], Varela ML[1], Besio Moreno M[1], Pereyra EN[1], Pignataro OP[1,2].
[1] *Institute of Biology and Experimental Medicine (IBYME-CONICET).* [2] *Department of Biological Chemistry, School of Sciences, University of Buenos Aires. E-mail: triniraices@hotmail.com*

Curcumin (CUR) or diferuloylmethane is a bioactive component of turmeric, a food additive derived from the rhizome of the plant *Curcuma longa*. This dietary polyphenol is the subject of countless studies since it has proven to have both prophylactic and therapeutic effects on illnesses as diverse as cancer, diabetes, cardiomyopathies, chronic pain and neurodegenerative diseases. One of its mechanisms of action is the activation of antioxidant pathways. Given that there is not much information about how it affects the endocrine population of the testis, the Leydig cells (LCs), we aimed to describe CUR effects on MA-10 tumoral cell line steroidogenesis and proliferation. On the other hand, we sought to clarify its interaction with one of the most important antioxidant enzymes, heme oxygenase-1 (HO-1). After a 5-h incubation with growing concentrations of CUR, we assessed progesterone production by radioimmunoassay and found that CUR stimulates LCs steroidogenesis at 20-40 µM concentrations and inhibits steroidogenesis at 80-100 µM. This inhibition correlates with a marked alteration of cell morphology and a significant decrease in cell viability as evaluated by trypan blue exclusion assay. Secondly, cell proliferation was assessed after a 24-h incubation period using the sulphorhodamine B assay. CUR has a positive effect at 20 µM concentration and decreases proliferation at 80-100 µM. We have previously described HO-1 negative effect on MA-10 steroidogenesis and proliferation. Cells were incubated for 5 h with CUR and subjected to Western blot assay. CUR augments basal HO-1 protein levels in a concentration dependent fashion. CUR also enhances HO-1 expression when stimulated with hemin, its inducer. What's more, if LCs are preincubated for 1 h with CUR and then subjected to a 5-h incubation with hemin, CUR can partially revert HEM negative effect on steroidogenesis provided that hemin does not induce HO-1 to such extent that it completely abrogates steroidogenesis regulation. The results presented herein contribute to the understanding on how curcumin regulates MA-10 cells functions and how it can impair them if present in high concentrations. Besides, we show that depending on how strong the stimuli upregulating HO-1 expression is, CUR can act as a protective agent reverting the negative effect of this enzyme on steroidogenesis.

A24

EFFECT OF BENZOPHENONES 2 AND 3 ON INSULIN SECRETION REGULATED BY AUTOPHAGY MODULATORS IN PANCREATIC BETA CELL LINE MIN6B1

Szulak F[1], Fernández MO[2], Becú-Villalobos D[1], Sorianello E[1].
1 - Laboratorio de Regulación Hipofisaria. 2 - Laboratorio de Neuroendocrinología. Instituto de Biología y Medicina Experimental (IBYME) – CONICET.E-mail: esorianello@dna.uba.ar

Benzophenones are present in cosmetics, plastics and otherindustrialgoods to protect human skin or products against direct exposure to ultraviolet (UV) radiation. Benzophenone-2 (BP2) and oxybenzone (benzophenone-3 or BP3) are common ingredients in sunscreen and have proven to be endocrine disruptors. Autophagy is an evolutionary conserved cellular self-degrading mechanism. It involves degradation and recycling of cellular components and plays an important role in cell homeostasis. Recent data reveal that benzophenones alter the autophagy process. The aim of the present study is to evaluate the effect of BP2 and BP3 on pancreatic beta cell function, more precisely, on insulin (INS) secretion in the presence of autophagy modulators.For this purpose a murine pancreatic beta cell line MIN6B1 was cultured in the presence of 10^{-5}M BP2 or BP3, or DMSO as a control, during 24h. In order to modulate autophagy, some groups were also incubated with Rapamycin (RAPA, autophagy inductor) or Chloroquine (CQ, autophagy flux inhibitor), alone or in combination with BP2 and BP3. Conditioned media were collected, centrifuged and cleared supernatants were subjected to a radioimmunoassay (RIA) for insulin determination.Our results show that BP2 alone did not change INS secretion in MIN6B1 beta cell line in comparison to the control condition while BP3 significantly inhibited INS secretion ($p<0.02$, n=11). The effect of BP2 or BP3 in the presence of the autophagy modulating compounds RAPA and/or CQ was then tested. RAPA induces autophagy by inhibiting autophagy inhibitor Mammalian Target of Rapamycin (mTOR). On the other hand, CQis a lysosomal inhibitor and triggers autophagy flux arrest. RAPA (50 nM) and CQ (10 µM) inhibited INS secretion as previously reported (ANOVA RAPA orCQ vs control $p<0.001$, n=13). Combination of autophagy modulating treatments with the benzophenones showed that BP2 partially reverted the inhibitory effect of RAPA (ANOVA RAPA vs Control $p<0.01$, RAPA-BP2 vs Control ns, RAPA-BP2 vs RAPA ns, n=7), but did not affect the inhibitory effect of CQ. BP3, on the other hand, did not alter INS secretion inhibition of RAPA (ANOVA RAPA vs Control $p<0.01$, RAPA-BP3 vs Control $p<0.01$, RAPA-BP3 vs RAPA ns, n=7) or CQ (ANOVA CQ-BP3 vs CQ ns, CQ vs Control $p<0.0001$, CQ-BP3 vs Control $p<0.0001$, n=11).We conclude that BP3 inhibits INS secretion in MIN6B1 pancreatic beta cells. In addition, BP2 but not BP3 reverts the inhibitory effect of RAPA, suggesting that benzophenones could modulate the autophagic process in pancreatic beta cells. This work was supported by CONICET, ANPCyT, Fundación René Barón, and Fundación Williams.

ANIMAL AND PLANT BIOLOGY AND BIOTECHNOLOGY I

A25

THE POTENTIAL OF RED SEAWEED MEAL AS FEED ADDITIVE FOR PRAWN *Artemesia longinaris*

Arzoz NS, Marcoval MA, Díaz AC, Espino ML, Velurtas MS, Fenucci JL.
Laboratorio de acuicultura (IIMyC – CONICET).E-mail: natiarzoz@gmail.com

Artemesia longinaris is a species of commercial interest distributed in the Southwestern region of the Atlantic Ocean from Southern Brazil to Patagonia (23-50 °S). Under culture conditions juveniles are kept in ponds at less than 2 m deep, so they are exposed to extreme environmental conditions like ultraviolet radiation. It has been shown that many species of red seaweed synthesize high concentrations of photoprotective compounds (PPCs) like UV absorbing compounds and carotenoids and these may constitute an interesting alternative in aquaculture feeds. The aim of this study was to determine the effects of a diet added with red seaweed meal of the family *Halymeneaceae* on physiological state of *Artemesia longinaris.* An experiment of 45 days was carried out on juveniles of *A. longinaris* (1.84±0.46g) obtained from the coastal waters of Mar del Plata Argentina (38°S 57º 33'W). Individuals were placed in 150L glass aquaria and fed every day with three isoproteic and isolipidic diets (45% protein, 8% lipid, 7% water, and 7% ash) with 0 (D), 1 (D1) and 2% (D2) of red seaweed meal. Each diet was tested in five replicated groups of six prawns each one. At the end of the experiment, survival, percentage of increase in weight, metabolites in serum haemolymph and PPCs concentration in tegument were determined. The survival varied between 50 and 76.6%, recorded the highest value in D2 treatment, although there were no significant differences between other treatments. The percentage increase in weight was 35% for individuals fed with diets D and D2 and 26% for those fed with diet D1. For metabolic variables, there was a significant decrease ($p< 0.05$) in cholesterol and triglycerides, in animals fed with added diets D1 and D2 respect to the animals fed with diet D. UV absorbing compounds only were detected in animals of treatment D1 and D2 with concentration of 0.16 (±0.03) OD g^{-1} and 0.23 (±0.08) OD g^{-1} respectively, but no significant differences were found. Carotenoids concentration show significant differences between treatments and recorded the highest values in treatment D2 (17.9 ±6.9 µg g^{-1} dry weight ($p< 0.05$). These results show that the use of red seaweed meal is feasible as additive in diets for *A. longinaris*. The recommended concentration should be 2% that which contributes to a better physiological state.

A26

PHOTOPROTECTIVE EFFECTS OF DIETARY CANTHAXANTHIN IN *Artemesia longinaris*

Espino ML, Arzoz NS, Marcoval MA, Díaz AC, Velurtas MS, Fenucci JL.
Laboratorio de acuicultura (IIMyC – CONICET).E-mail: natiarzoz@gmail.com

Artemesia longinaris is a species of commercial interest distributed along the South American coast (23-50°S). During the last decades, it has shown an increase in ultraviolet radiation (UVR) above Earth's surface. While penaeoid shrimps are coastal species that inhabit generally at depths greater than 10m, under cutivation conditions are kept in ponds at 2 m deep, so they are exposed to extreme environmental conditions. Carotenoids are considered bioaccumulative photoprotective compounds in integument of crustaceans, which must be incorporated with diet. Although astaxanthin (Ax) is the main carotenoid, decapods are able to synthesize it from other carotenoids, e.g. canthaxanthin. The aim of this study was to determine the bioaccumulation of carotenoids from a diet added with canthaxanthin on juveniles *A. longinaris* and its possible protective effects under conditions of stress by UVR. Previous to the experiment with radiation, prawns (1.5±0.3g) were placed during 5 weeks in individual aquaria under controlled conditions of temperature, pH, salinity and photosynthetically active radiation (PAR) under three feeding treatments. One group fed with basal diet (B) (45% protein, 8% lipid, 7% water and 7% ash) and others with 100 (C_{100}) and 300 (C_{300}) mg canthaxanthin Kg^{-1} diet. After 4 weeks animals were subjected to two radiation treatments, by triplicate: B+PAR (400-700nm), C_{100} or C_{300}+PAR (400-700nm), and C_{100} or C_{300}+UVR (280-700nm). Survival varied between 83-100% for PAR treatment, under stress by UVR the C_{300} treatment showd the same survival than PAR treatment (83%). Concentration of β-carotene and both, free and esterified Ax forms, were significantly higher in prawns fed with C_{100} and C_{300}+PAR (β-carotene: 5.51±1.01 and 5,63±0,57; free Ax: 2.73±0.51 and 2.78±0.88; esterified Ax: 1.88±0.49 and 1.22±0.49µg g^{-1}, respectively) than B+PAR (β-carotene: 2.86±0.92; free Ax: 1.25043; esterified Ax: 1.04±0.26µg g^{-1}). There were no significant differences between C_{100} and C_{300}+UVR (values varied between β-carotene: 1.44-1.07; free Ax: 0.78-0.77µg g^{-1}), but these values were significantly lower than those recorded for C_{100} and C_{300}+PAR, except for esterified Ax (0.58-0.67µg g^{-1}). It can be concluded that canthaxanthin acts as a protector of UVR stress in *A. longinaris*, through biotransformation and bioaccumulation of carotenoids.

A27

MITOCHONDRIA RAT LIVER PEROXIDATION: EFFECT OF A METHANOLIC EXTRACT OF *Cestrum parqui* L´HERIT

Barberón J, Becerra V, Ventura M, Leaden P, Leggieri M, Zeinsteger P, Palacios, A.Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.E-mail: jbarberon@fcv.unlp.edu.ar

Cestrum parqui L´Herit is a poisonous plant of the Solanaceae family that affects large animals when they consume the leaves. The toxic compound is an atractyloside that inhibits the ADP/ATP carrier leading to ATP depletion that affects cells, especially hepatocytes, being coagulative necrosis the main lesion. Besides the toxic compound other active principles are present in the plant. Aim of this study was to investigate the effect of *C. parqui* methanolic extract on the peroxidation of mitochondria membranes of hepatocytes. Rat liver mitochondria where incubated with a methanolic extract of the plant in an *in vitro* non-enzymatic ascorbic acid-Fe^{+2} system in order to determine the oxidative effect on membranes and to quantify peroxidation level in standardized conditions. Different concentrations were used (0.05, 0.10, 0.20, 0.40, 0.80, 1.6 mg) to determine the effect of peroxidation on membranes, which was determined by means of chemiluminescence in cpm (counts per minute). Membranes without extract and with only ascorbic acid were used as controls. The methanolic extract of *C. parqui* demonstrated to have oxidizing effect which was concentration dependent. It has been demonstrated that many chemicals show toxicity in an increasing concentration-dependent manner and that the route of entry is important as well. In the case of *C. parqui*, cattle can ingest the leaves during the winter season due to hunger, leading to hepatotoxicity due to the atractylosides present in the plant.

A28

FORMULATION OF ADAPTED DIETS FOR *IN VIVO* STUDIES OF CHRONIC NONCOMMUNICABLE DISEASES

Colussi NA[1], Romero Vidomlansky PR[1], Todaro JS[1], Melana Colavita JP[1], Acevedo BA[2], Chaves MG[2], Rodríguez JP[1], Aguirre MV[1].
[1]Laboratorio de Investigaciones Bioquímicas de la Facultad de Medicina (LIBIM) de la Universidad Nacional del Nordeste.
[2]Grupo de Investigación en Química Aplicada (GIQAP) de la Facultad de Ciencias Exactas y Naturales de la Universidad Nacional del Nordeste.E-mail: narellacolussi@gmail.com

The laboratory mouse is a preeminent study tool in modern experimental research, natural food ingredients such as chia seeds (*Salvia hispanica*), rich in essential fatty acids represent an innovative alternative for the production of food destined to murine.The objective was to formulate foods rich in essential fatty acids from crushed chia seeds and various protein sources for the establishment of experimental models applicable in studies of chronic non-communicable diseases. Male mice of the Balb-c strain (2 months old)of the Faculty of Medicine Bioterium were randomly distributed in four lots (4 animals each) and provided with water and food *ad libitum.* Lots were identified as Control group 1= diet A (commercial balanced X); Control group 2= diet B (commercial balanced Y); Experimental group 1= diet C (crushed chia seeds, fish thorn and crushed cowpea (*Vigna unguiculata*), experimental group 2= diet D (crushed chia seeds and skimmed milk powder). Diets C and D were designed according to the nutritional requirements of Balb-c

mice. Pellets were made handcrafted according todry heat sterilization (80°C-120')/microwave (100g-1'-1425 MHz)/UV irradiation (254nm-15') seeking the optimal conservation and maintanance of good texture. The animals were fed for 1 month and were monitored weekly by weighing and direct observation. Consumed food was also measured. The average weight of mice from lots C and D was 28.6±1.9 g and 28.4±1.9 g. Weekly average of food consumption was 28.2±5.2 g for diet C and 23.5±5.2 g for diet D, framed in normality. Biochemical determinations were assessed from blood samples obtained by cardiac puncture under anesthesia. Mice fed with diet C showed: cholesterolemia 57.7±2.86 mg/dl (RV 63-174 mg/dl), glycemia 113.7±30 mg/dl (RV 106-278 mg/dl) and triglyceridemia 76.2±17.7 mg/dl (RV 71-164 mg/dl). Finally, this study revealed that the diet C formulation was accepted preferably by mice and confers them favorable health conditions. Additionally, the most efficient technology for pellets preservation was UV irradiation.

A29
ISOLATION AND PARTIAL PURIFICATION OF A NOVEL THERMOSTABLE TRYPSIN INHIBITOR FROM CHAÑAR (*Geoffroea decorticans*) SEEDS

Cotabarren J[1], Broitman D[1], Melchiori P[1], Pizzano M[1], Quiroga E[2], Obregón WD[1]
[1]Centro de Investigación de Proteínas Vegetales (CIPROVE-CIC-UNLP).[2]Laboratorio de Membranas y Biomateriales. Instituto de Física Aplicada (INFAP), CONICET-UNSL.E-mail: cotabarren.juliana@biol.unlp.edu.ar

Plants are good sources of protease inhibitors (PIs), which protect them against diseases, insects, pests and herbivores. PIs are very important signaling molecules that regulate processes such as inflammation, apoptosis, blood coagulation, cardiovascular diseases and neurological disorders. In the case of serine protease inhibitors, they are widely studied due to their large number of biological properties; anticoagulant, antimicrobial, etc. The seed of chañar *(Geoffroea decorticans)* is well known and it has been previously investigated for its chemical composition and nutritive properties. Even though the protein enzyme inhibitors are being extensively research due to their important functions, there have been no previous systematic investigations demonstrating the presence of proteases inhibitors in the seed of chañar. Moreover, there are no reports to date of protein fractions studied from *Geoffroea decorticans*. In this study we report the isolation and partial purification of the first trypsin inhibitory fraction isolated from *Geoffroea decorticans*, which we call GdTI. The initial crude extract was obtained by blending chañar´s seeds with addition of 0.1 M Tris-HCl buffer, pH 7.4, and clarification by centrifugation. The supernatant was partially purified by heat treatment at 90 °C during 30 min and subsequent affinity chromatography using a matrix of Trypsin immobilized on glyoxyl-agarose. The purification steps were evaluated by studying the protein profile by SDS-PAGE and protein quantification by the Bradford´s method. GdTI exhibited molecular masses of 6.5 and 20 kDa (MALDI-TOF/MS) demonstrating the presence of two isoinhibitors. The IC50 value was 1.5 µg/ml. In addition, we demonstrated the stability of GdTI to temperature by incubation at 40, 50, 60, 70, 80, 90 and 100 °C during 60 min, and stability to pH 2, 4, 6, 8, 10 and 12 during 60 min. This study represents the first report of a trypsin inhibitory fraction isolated from *Geoffroea decorticans.* Our results encourage the analysis of GdTI´s potential biological activity, as antioxidant, anticoagulant and antimicrobial agent in order to exploit its biotechnological applications.

A30
EFFECT OF *Silybum marianum* L. ON OXIDATIVE DAMAGE IN CELLULAR MEMBRANES OF CHICKEN LIVER

Gavazza M, Marmunti M, Ventura M, Leaden P, Savignone C, Zeinsteger P, Palacios A.
Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata.
E-mail: belenventura87@gmail.com

In living beings, antioxidants are of vital importance for protection against oxidative damage produced by reactive oxygen species. Silymarin, present in extracts of the plant "cardoasnal" (*Silybum marianum* L.*)* is composed of four flavolignans of similar structure, and has a recognized hepatoprotective effect. In this work, its antioxidant effect *in vitro* on non-enzymatic peroxidation in mitochondria and microsomes chicken liver was studied. A commercial preparation was used for supplementation in drinking water of poultry and pigs composed of 16% silymarin phosphatide. Oxidative stress on organelles was induced by submitting the samples (1 mg of protein) to a Fe^{+2}-ascorbate dependent pro-oxidant system at 37 °C. Oxidative damage was quantified by chemiluminescence using a Packard1900 TR liquid scintillation counter (Meriden CT, USA). The chemiluminescence expressed as cpm (counts per minute) was read every 10 minutes to establish the course of the peroxidation as a function of time. Likewise, the value of total cpm (summation of the readings) was used to compare the inhibitory effect of the commercial preparation using different concentrations corresponding to 6.25; 12.5 and 25 µg of the active principle (silymarin phosphatide) per mg of mitochondrial and microsomal protein. Controls were performed simultaneously without the addition of ascorbate. Inhibition of peroxidation was concentration dependent on silymarin in the membranes studied. The results show that for all thetested concentrations, a protective effect on the oxidative injury induced was found.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

NEW TECHNOLOGIES I

A31
CLINICAL EVALUATION OF LINGUAL FRENECTOMY IN PATIENTS TREATED WITH CHIRURGICAL NEAR INFRARED DIODE LASER

Gatti DB, Llasen MJ, Bonino AM, Marchese Ragona CC, Higuera J.
E-mail: consultdbg@hotmail.com

Ankyloglossia (tied tongue) is a disorder present in 0.1 to 12.11% of infants where the lingual frenum, a band of fibrous, muscular or mixed connective tissue covered with a mucous membrane in the midline, joining the tongue with the floor of the mouth with an anomalous insertion, which can be Light (Class I, 12 to 16 mm), Moderate (Class II, 8 to 10 mm), Severe (Class III, 3 to 7 mm) or Complete (Class IV (3 mm or less) according to the classification of Kotlow. Ankyloglossia can affect breastfeeding, phonation, swallowing and normal development of the jaws. The conventional surgical technique in these cases consists in the elimination with a scalpel or scissors of the frenulum, with abundant hemorrhage and subsequent suture. In some cases, general anesthesia is needed. Near infrared diode laser is cited as an option for frenectomy, presenting advantages and simplicity in the trreatment of Thaís patology. The aim of this study is to evaluate the presence of intraoperative hemorrhage, the clinical appearance of tissues treated with and without laser at 3 and 7 days, presence of absence of postoperative pain and immediate lingual mobility after surgery with both techniques.

A32
FIRST STEPS TO DEVELOP A DETECTION METHOD OF *Brucella* SPP., APPLYING THE MOLECULAR TECHNOLOGY OF ISOTHERMAL AMPLIFICATION

Hermida P[1], Larocca L[1], Stolowicz F[1], Werbajh S[1], Zorreguieta A[2], Vojnov A[1], Carrillo C[1].
1- Instituto de Ciencias y Tecnología Dr. César Milstein – CONICET.2- Fundación Instituto Leloir.
2- E-mail: ccarrillo@centromilstein.org.ar

Brucellosis is a chronic infectious disease that has a big impact on veterinary and human health. This zoonosis is caused by bacteria of the genus *Brucella* spp., with a strong affinity fo rreproductive organs, making sexually mature and pregnant mammals more susceptible to infection. Currently, the disease can be diagnosed either: i- directly by blood, marrow or other tissue cultures; or ii- indirectly through a serological reaction, being more frequently chosen because it is simpler and safer than the first one. Our purpose is to develop new detection methods for Brucellosis with highly specific and sensitive molecular techniques such as PCR. Yet, PCR and other similar techniques have one major drawback: they need to be performed at a lab with specific infrastructure and trained personnel. Thus, our main goal is to develop and set up a Loop mediated isothermal amplification (LAMP) to detect *Brucella sp*., which should share the benefits of PCR but needing just a simple heater. Three primer sets were designed with a specific software (LAMP Design) and tested in a LAMP basic reaction using DNA from *B. abortus*' (human pathogen) as a template. The three sets were functional, with different sensitivity and specificity parameters. Later, we adjusted the reaction components, designing a mix that avoids having to manipulate various reagents; while all three sets maintained their functionality. Afterwards, the optimal conditions (time and temperature) of the reaction were determined for each primer set, finding a range of temperature from 62 to 66°C and 50 to 60 minutes of time. Finally, we developed a simple result-reading method with a sensitivity comparable to that of the analytical method of agarose gel electrophoresis.These results encourage us to continue working and test artificially-inoculated samples, and subsequently test clinical samples, to bring us closer to the development of a simple and effective test kit to detect Brucellosis.

A33
COLLAGEN HYDROGELS FOR DEXAMETHASONE PHOSPHATE-CONTROLLED DRUG RELEASE

Ibar A, Galdopórpora JM, Morcillo MF, Tuttolomondo MV, Desimone MF.
IQUIMEFA-CONICET. Facultad de Farmacia y Bioquímica, Universidad de Buenos, Aires, Junín 956 Piso 3, (1113) Ciudad Autónoma de Buenos Aires, Argentina.E-mail: jgaldoporpora@ffyb.uba.ar

The development of biomaterials has attracted great attention for wound healing and medical purposes. Dexamethasone phosphate is a widely used anti-inflammatory drug. To provide a proper support to this anti-inflammatory agent a protein scaffold was tested. Collagen is a well-characterized protein used in the biomedical field because of its biological origin, biodegradability, biocompatibility and ease of obtention. SEM images show collagen typical fibrillar network and their striation. Dexamethasone phosphate release from the collagen hydrogel dressing is described by a biphasic model probably due to encapsulation in microdomains. The first plateau is reached at 6 hours with 60% of total dexamethasone phosphate content release and the second plateau is reached at 48 hours with 100% of total dexamethasone release due to collagenase proteolytic action. A diminish in weight is seen due to collagenase action with a loss of 50% of its initial weight at 24 hours and a complete loss of weight at 48 hours. DSC profile, FTIR spectra and biphasic

kinetics release showed that the main interactions between the material and the drug are electrostatic and intrapore. DSC profile showed no difference between the two hydrogels. FTIR spectra for collagen-dexamethasone showed typical absorption bands in collagen. To identify the drug-polymer interaction, a peak at 890 cm^{-1} (NH_2 wagging) disappearance was observed due to amine and phosphate electrostatic interaction. Collagen-dexamethasone hydrogel showed good biocompatibility with MDBK cell line and a swelling ratio of 300%. High swelling ratio and a cell survival of near 100% at 24 hours and 190% growth at 48 hours lead us to believe this material has clear advantages for biomedical applications. To summarize, a novel biocompatible hydrogel for tissue engineering was synthesized.

A34
DELIVERY OF DOXORUBICIN FROM MAGNETIC NANOTHERANOSTICS: IMPROVEMENT OF THE ANTITUMORAL EFFECT REGARDING THE ADMINISTRATION OF FREE DRUG IN COLORECTAL TUMOR DERIVED CELLS

Martín MJ[1,2], Azcona P[1], Gentili C[2], Lassalle V[1]
[1]INQUISUR, Departamento de Química, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina
[2]INBIOSUR, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina.E-mail: ma.julia.martin@gmail.com

The incursion of magnetic nanoparticles (MNPs) in the biomedical field has derived in interesting and more efficient tools to resolve traditional problems. The interest is especially centered in the diagnosis and treatment of oncological diseases mediated by the targeted delivery of drugs. The MNPs are, in general, composed of a magnetic core, commonly of iron oxides, modified with different substrates that improve the "active targeting". Folic acid (FA), is a widespread molecule used to assess active targeting because of its high affinity for the folate receptors (FR) that are overexpressed on the surface of many human cancer cells, including colorectal cancer (CRC) cells. In statistical terms, CRC is one of the most important causes of morbidity and mortality worldwide and it is the second most frequent type of cancer in Argentina according to the National Cancer Institute. Doxorubicin hydrochloride (DOX) is a broad-spectrum fluorescent antitumor drug for the treatment of CRC and other types of cancer. DOX presents high systemic toxicity, meaning that it is unable to discriminate between cancer and normal cells subjected to rapid division. The targeting of this drug by MNPs could result in an increased bioavailability and a reduction in secondary toxicity. The objective of this work is to provide knowledge about the interactions between the theranostic agent composed of magnetite, FA and DOX and cultures of the cell line derived from CRC, HCT116. The effects on cell viability were evaluated by means of trypan blue dye exclusion test, revealing that the treatment with DOX-Mag for 24 hours significantly decreased the number of living cells with respect to the same doses of free drug. The influence of MAG or FA (in the absence of the drug) on this anti-proliferative effect was ruled out. The effective internalization of MNPs was qualitatively confirmed by staining with Prussian Blue. The quantification of intra and extracellular iron was assessed by absorption atomic spectroscopy by iron determination. In addition, taking advantage of the fluorescence of the DOX molecule, dynamic studies of the release of the drug from DOX-Mag within the cells were performed by fluorescence microscopy. Finally, we purpose that the enzymatic activity over MNPs improved the drug release respect to the values obtained in phosphate buffer at different pH. Taken together, these data suggest that the selectivity given by the FA in the coating of these nanosystems markedly increased the effect of doxorubicin on human CRC models. In this context, this contribution expands the knowledge of the behavior of nanocarriers in contact with *in vitro* models and proposes the DOX-Mag as a potential theranostic agents for improvement of cancer treatment.

A35
STUDY OF THE INTERACION BETWEEN CELLS AND SILICA NANOPARTICLES WITH DIFERENT SIZE AND SURFACE

Mebert A[1,3], Evelson P[2], Desimone M[1], Maysinger D[3].
[1]Instituto de la Química y Metabolismo del Fármaco (IQUIMEFA), [2]Instituto de Bioquímica y Medicina Molecular (IBIMOL) Facultad de Farmacia y Bioquímica. Buenos Aires, Argentina and [3]Faculty of Medicine, McGill University, Montreal, Canada.
E-mail: amebert@ffyb.uba.ar

Silica nanoparticles (SiNPs) are currently being used in cosmetic products, food, paints, and other industries and is widely believed to be an important material for biomedical and drug delivery applications. They can be produced using synthetic techniques with precise size control and physical and chemical properties. Human exposure to nanosilica can occur unintentionally in daily life and in industrial settings. With the aim of studying its interaction with human body different size (60 nm, 100nm and 300 nm) SiNPs were synthesized by Stöber method and surfaced grafted (bare -OH, amino -NH_2 and thiol -SH) with organosilane chemistry using (3-aminopropyl)triethoxysilane (APTES) and (3-mercaptopropyl)trimethoxysilane (MPTMS). In the present work, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were performed to evaluate the cytotoxicity of these NPs in cervix (HeLA), lung (A549) and brain (U251) cell line cultures. To determine nanoparticles fate after cell exposure, fluorescent rhodamine labeled 60 nm silica particles were synthesized and grafted with APTES or MPTMS to obtain amino modified and thiol modified fluorescent labeled particles. Size, dispersity and surface were studied by scattering electronic microscopy (SEM) as well as the hydrodynamic diameter by dynamic light scattering (DLS) and surface grafting by fourier-transform infrared spectroscopy (FT-IR).

Results show that no evident cytotoxicity in the tested concentration and time, up to 30 ppm and 72 h. Internalization studies shown that those NPs grafted with positively charged groups were more capable to enter the cells.

A36
ANTIMICROBIAL AND ANTI-INFLAMMATORY EFFECTS OF DODECENYLSUCCINIC ANHYDRIDE MODIFIED COLLAGEN HYDROGELS LOADED WITH SIMVASTATIN

Olivetti CE, Alvarez Echazu MI, Perna O, Mitarotonda R, De Marzi M, Desimone MF, Alvarez GS.
Facultad de Farmacia y Bioquímica *(FFyB-UBA), IQUIMEFA-CONICET e Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INEDES) CONICET-UNLu.E-mail: colivetti@ffyb.uba.ar*

Collagen dressings have been extensively used for wound healing as they restore the barrier function lost during injury, while stimulating cell migration and supporting the growth of new cells in the affected area. Nevertheless, collagen scaffolds often present poor mechanical properties, high susceptibility to degradation and low capacity to incorporate hydrophobic drugs. In order to improve their properties, physical and chemical modifications are necessary. Herein, we developed a novel hydrophobic collagen wound dressing to incorporate simvastatin, which has potential application in the treatment of ulcers and prevention of wound infection, among other properties such as antioxidant, anti-inflammatory, and antifibrotic effects as well as angiogenic activity. For that matter, collagen hydrogels were grafted with dodecenylsuccinic anhydride (DDSA). Solid NMR was performed in order to confirm that DDSA was covalently bound to hydroxyl or amine groups of collagen amino acids. Also, an increase in the water contact angle was observed, providing good evidence of the chemical modification proposed for the reactive, and the incorporation of hydrophobic domains. Modified hydrogels were loaded with simvastatin showing higher adsorption capacity and lower release. Lastly, the antimicrobial and anti-inflammatory activities of DDSA-collagen materials were assessed. DDSA-collagen hydrogels, either unloaded or loaded with simvastatin showed sustained antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* for 72 h probably due to the hydrophobic interaction of DDSA chains with bacterial cell wall. The antimicrobial activity was stronger against S. *aureus*. Collagen hydrogels also presented a prolonged antibacterial activity when they were loaded with simvastatin, confirming the antimicrobial properties of statins. Finally, it was observed that these materials can stimulate resident macrophages and promote an M2 profile which is desirable in wound healing processes.

A37
VALIDATION OF SILICA-COLLAGEN NANOCOMPOSITE FOR PROLONGED GROWTH HORMONE DELIVERY

Villarruel LA, Brie B, Scilletta NA, Desimone MF, Becú-Villalobos D, Catalano PN.
Departamento de Micro y Nanotecnología (INN, CNEA-CONICET), Instituto de Química y Metabolismo del Fármaco (UBA-CONICET) y Laboratorio de Regulación Hipofisaria (IBYME-CONICET). E-mail: luisvillarruel@cnea.gov.ar

Pharmacological treatments with growth hormone (GH) in short stature syndromes require daily injections and therefore new formulations are needed. In this context, we have analyzed how surface modifications of silica nanoparticles (SiNP) allow loading of GH to be tested as a prolonged hormone delivery system in a mouse model (neuroDrd2KO) in which continuous GH secretion feminizes hepatic gene expression. We previously demonstrated that unmodified SiNP offer a GH higher adsorption capacity (12 ng GH/ng SiNP) compared to chemically modified ($–NH_2$, –SH, isobutyl) SiNP. These GH-loaded SiNP showed a progressive GH release profile reaching 0.3 ng GH/ng SiNP after 5 days. In the present work we sought to validate GH release kinetics from two delivery systems: GH-loaded SiNP-collagen nanocomposite (GH@SiNP@collagen) and GH-loaded on collagen matrix (GH@collagen) were tested as prolonged GH delivery systems. To that end, 200 nm-diameter SiNP were prepared following the Stöber method and maximum GH adsorption was allowed by incubation with hormone for 24 h. To prepare GH@SiNP@collagen, GH-loaded SiNP were incorporated in a collagen matrix during its preparation. GH was also incorporated in a collagen matrix to prepare the GH@collagen system. Determination of GH release was assessed for up to 5 days by monitoring the hormone concentration in exposed media by RIA. Both systems showed a progressive GH release profile. GH@SiNP@collagen released 40% of GH loaded on SiNP reaching 0.1 ng GH/ng SiNP after 5 days. On the other hand, GH@collagen released 100% of loaded GH reaching 2.4 ng GH/ng SiNP after the same time period. These results indicate that GH@SiNP@collagen can be used to provide a prolonged GH delivery and that this nanocomposite is adequate to be tested on the dwarf neuroDrd2KO mouse model. *Supported by PFIZER, ANPCYT, Fund. BARON & Fund. WILLIAMS.*

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

MICROBIOLOGY

A38

EFFECTS OF CARBON SOURCES ON SECONDARY METABOLITES BIOSYNTHESIS BY *Fusarium verticillioides*

Achimón F, Jacquat A, Brito V, Dambolena S, Zygadlo J and Pizzolitto R.*
*Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET-UNC-Córdoba- Argentina) Instituto de Ciencia y Tecnología de los Alimentos (ICTA-UNC- Córdoba- Argentina) *E-mail: fachimon@imbiv.unc.edu.ar*

Fusarium verticillioides is one of the most important fungal pathogens of maize, being responsible of major economic losses worldwide due to its effect on grain quality and mycotoxincontamination, particularly fumonisin B_1 (FB_1), which is extremely harmful to human and animal health. Secondary metabolites (SMs) production often occurs during stationary phase. *F. verticillioides* SMs include toxins such as FB_1, volatile organic compounds such as sesquiterpenes (SQT) and pigments (naphthoquinones), among other metabolites. The aim of the present study was to determine the effect of different carbon sources, consisting of glucose, sucrose, lactose, fructose or xylose, on vegetative growth and secondary metabolism in *F. verticillioides*. For evaluation of growth, *F. verticillioides* M3125 was incubated at 28°C in Czapek Dox Agar (CDA) supplemented with different carbon sources. For evaluation of secondary metabolites, liquid GYAM medium was used (adjusted to pH 3) and cultures were incubated with shaking at 25°C for 7 days. At the end of the incubation period, pH value was registered, FB_1 quantification was performed using a HPLC, SQT quantification was carried out using a GC-MS and total naphthoquinones were quantified with a spectrophotometer. Lower lag phase values were achieved with lactose and sucrose as carbon sources (both disaccharides), compared to glucose, xylose and fructose (monosaccharides). This may be explained by the development of more extended andpoor branched hyphae when the fungus grows with disaccharides as carbon sources. This may be a strategyto explore the surrounding media for areas with simpler carbohydrates. The growth rate was higher with fructose and conidiation was higher with lactose. FB_1 biosynthesis was statistically higher with sucrose and glucose (both with pH 3.5) and lower with xyloseand fructose. On the other hand, naphthoquinone biosynthesis was statistically higher with fructose (pH 3.7) followed by xylose (pH 4.8), while lower values were achieved with glucose and sucrose. Lactose did not support the biosynthesis of these SMs. On the other hand, SQT biosynthesis was statistically higher with xylose compared to the other carbon sources. According to our results, growing parameters, conidiation and biosynthesis of secondary metabolites are regulated by both environmental (pH) and nutritional factors (carbon source). Also, our results suggest an inverse relationship between FB_1 and naphthoquinones biosynthesis. It is well documented that both metabolites proceed via the polyketide route by formation of a common precursor. In addition, there are global and specific regulators that respond to environmental signals and activate one of the metabolites while repressing the other.SQT production does not show a pattern with the other SMs studied. In fact, its biosynthesis proceeds through a different pathway, the mevalonic acid pathway. It has been proposed that trichodiene synthase activity (the enzyme involved in the first step of SQT biosynthesis) is optimum at pH values near 6, which could explained the higher amount of SQT produced with xylose as carbon source (pH 4.8). However, xylose itself could represent a signal that induces SQT biosynthesis.

A39

EFFECT OF GLUCOSE CONCENTRATION ON GROWTH AND FATTY ACIDS PROFILE OF TWO *Rhodotorula glutinis* STRAINS

Angelicola MV[1], Viñarta SC[1,2,3], Aybar MJ[2], Figueroa LIC[1,4].
[1]Planta Piloto de Procesos Industriales Microbiológicos (PROIMI)-CONICET. S. M. de Tucumán, Argentina. [2]Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT, e Instituto de Biología, FBQF, UNT. S. M. de Tucumán, Argentina. [3]Microbiología, Facultad de Ciencias Exactas y Naturales, UNCA. S. F. del Valle de Catamarca, Catamarca, Argentina. [4]Microbiología Superior, FBQF, UNT. S. M. de Tucumán, Tucumán, Argentina.
E-mail: mv_angelicola@hotmail.com

Certain oleaginous yeasts as *Rhodotorula glutinis* can accumulate neutral lipids up to 70% of dry biomass, consisting mainly in triacylglycerols (TAG), under appropriate cultureconditions. Microbial TAG represents a valuable alternative feedstock for biodiesel production. Lipid accumulation occurs under nutrient limitations, mainly nitrogen, with simultaneous excess of carbon source.The lipid synthesis andfatty acids (FA) composition are influenced by several factors as carbon source (type and concentration), aeration, growth, temperature, C/N ratio, pH, among others parameters. Effect of initial glucose concentration (30, 40 and 100 gL^{-1}) on growth, lipid synthesis and FA profile of two strains of *R. glutinis* (R4 and R48) isolated from Antarctica was investigated using a nitrogen-limited medium (MI). Yeasts were incubated aerobicallyon a rotatory shaker during 120 h at 250 rpm and 25 °C. Analytical determinations (biomass, lipid production, lipid content and residual glucose) were performed after 120 h of culture time. Glucose affected the growth of the strains and the FA profile of the microbial TAG. According to the results, the growth exhibited by R48 (12.33-13.83 g L^{-1}) were higher than R4 (8.42-10.83 g L^{-1}) for all glucose concentrations assayed. An increase of the lipid content (from 50.6 to 60.2%) and a decrease of the biomass were exhibited by R4 when the glucose concentration increased from 30 to 100 g L^{-1}. However, significant differences on lipid accumulation and growth were not observed for R48 ($p > 0.05$, Tukey test).The FA profile of microbial lipids was analyzed by GC-FID.FA with chain length between 14 and 18 carbons dominated the lipidic profile of

both strains. Eighteen-carbons FA were the most abundant (68.27-85.59%). The increase of glucose concentration affected the FA composition of the yeasts. Higher glucose concentrations caused an increase in the relative abundance of oleic and palmitic acids with a simultaneous decrease of linoleic and linolenicacids in in the FA content of R48 strain. Different effect of glucose concentration was observed in R4 and a decrease of oleic and linoleic acids with an increase of palmitic acid were observed at the highest glucose concentration. Results demonstrate that the FA profile of *R. glutinis* R4 and *R. glutinis* R48 are comparable with other oleaginous yeasts reported inthe literature. The better FA composition as feedstock for biodiesel was observed by R4 with 30 g L^{-1} of glucose, whilst for R48, with 100 g L^{-1}. The FA profile of *R. glutinis* R4, with a high content of oleic acid (~60%), is quite similar to vegetables oils, indicating that its lipids have potential as a feedstock for biodiesel production.

A40
OXIDATIVE STRESS RESPONSE OF NATIVE YEASTS TO HEAVY METALS

Bernal A[1], Polito F[1], Cruz E[1], Figueroa LIC[1, 3], Fernández P[1 ,2], Nieto Peñalver C[1, 3]
[1] PROIMI-CONICET, San Miguel de Tucumán, Tucumán, Argentina;[2] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, Argentina;[3] Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina.
E-mail: anahirbernal@gmail.com

A possible consequence of heavy metal exposure is an increased production of reactive oxygen species (ROS) that could induce or exacerbate intracellular oxidative stress and can cause physiological stress. Defense mechanisms which counteract the impact of ROS, including enzyme and non-enzyme antioxidant systems, are found in all aerobic cells. In this work we study the antioxidant response of native yeasts to the oxidative stress produced by heavy metals.Yeast strains *Meyerozyma guilliermondii* 6N and *Rhodotorula mucilaginosa* 7Apo1 were cultured in YM media in absence/presence of $K_2Cr_2O_7$ and$CuSO_4$ alone or in metal mixtures. Cell-free extract was prepared and antioxidant enzymatic activities [catalase (CAT), superoxide dismutase (SOD) and thioredoxinreductase (TrxR)] were determined. The specific activity is given as U/mg protein. Total antioxidant capacity was determined by the modified ABTS* discoloration method [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)]. In both strains, the activity of SOD was higher in the presence of Cr (VI) showing significant differences with the control condition without the metal ($p < 0.05$, Tukey test), while this activity was reduced in the presence of Cu (II) and the mixture of metals. For the CAT activity, in both strains the highest values were obtained with Cr (VI) and the mixture of metals but this activity was lower in the presence of Cu (II). In the case of the TrxR activity, 7Apo1 and 6N did not show significant differences in the assayed conditions ($p > 0.05$, Tukey test). The total antioxidant capacity of 6N with Cr (VI) and Cu (II) was higher than the control while no differences were observed in themixture of metals. 7 Apo1 did not show significant differences in the tested conditions ($p > 0.05$, Tukey test). Interaction between chemical elements, the level of oxidative stress and antioxidant defense play an important role in ecotoxicological response of microorganisms in polluted environments and our results provide additional confirmation for metal-mediated activation of the antioxidant enzymes in yeasts.

A41
FIRST MYCOVIRUS IDENTIFIED IN *Fusarium verticillioides* AND *Fusarium. andiyazi*

Jacquat AG[1], Iglesias J[2], Cañizares MC[3], Theumer MG[4], Dambolena JS[1], García-Pedrajas MD[3].
1: IMBIV (CONICET-UNC), 2: INTA-EEA Pergamino, 3: Laboratorio de Micología (IHSM-UMA-CSIC, España), 4: CIBICI (CONICET-UNC).

The fungal pathogen *Fusarium verticillioides* (Fv) is the main cause of ear rot of corn in Argentina and one of the major producers of the mycotoxin, fumonisins, which can cause health problems to humans and farm animals. The use of synthetic fungicides is the most used strategy to control the phytopathogenic fungi in plants. However, in view of the potential negative impact on the environment, new friendlier strategies such as biological control should be taken into account. The mycoviruses are intracellular infectious agents specific to fungal cells. These generally have double-stranded RNA genomes. Although it has not been reported that most of mycoviruses cause phenotypic changes in the host, some of them have been associated to phenotypic alterations including hypovirulence in phytopathogenic fungi. Due to this, the mycoviruses could be promising biocontrol agents to phytopathogenic fungi. Until today, there are no reference about mycoviruses isolated from Fv. The objective of this study was to determine the incidence of the mycoviruses in Fusarium spp. strains isolated from Argentina. One hundred monosporic isolates of Fusarium spp. were isolated from maize and sorghum in Argentina. The presence of dsRNA was evaluated using CF-11 cellulose chromatography. The obtained results demonstrated that among 100 isolates analyzed, 2 of them showed a monopartite dsRNA extrachormosomal bands (approximately 3.5 Kbp), after electrophoresis in 0.8% agarose. These genetic elements were vertically transmitted during conidiation and showed resistance to S1 nuclease and DNAseI degradation. The fungal host strains were molecularly identified as *Fusarium verticillioides* and *F. andiyazi*, according to sequence homology of the tubulin and transcription factor 1-alpha genes. These monopartite dsRNA extrachormosomal fragments were named as dsRNA-Sec505 y dsRNA-162, according to the fungal isolate from which they were identified. The monopartite dsRNA fragments found in this work are consistent with the mycoviruses previously described in other fusarium species, so this is probably the first report of mycoviruses isolated in *F. verticillioides* and *F. adiyazi* until date. Sequencing studies are being performed in order to identify and classify into taxonomic groups, the detected mycoviruses. The potential of these mycoviruses to be used as a strategy to control the phytopathogenesis and mycotoxigenesisof *F. verticillioides* and *F. andiyazi*, remains to be studied.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A42
EFFECT OF COPPER ON THE QUORUM SENSING SYSTEM OF *Pseudomonas capeferrum* WCS358

Leguina AC[1], Lacosegliaz M[12], Palacios EAR[12], Fernández PM[1], Castellanos de Figueroa LI[12], Nieto Peñalver CG[12].
[1]Planta Piloto de Procesos Industriales Microbiológicos PROIMI-Biotecnología CONICET.[2]Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán.E-mail: carolinaleguina12@gmail.com

In agriculture, copper has largely been utilized for the control of phytopathogen fungi. However, copper tends to accumulate in soils resulting in negative biological effects. Although copper is one of the most studied metals, its effect on microbial interactions mediated by Quorum Sensing (QS) between rhizosphere microorganisms has not been evaluated up to date. QS systems are signaling mechanisms that control the microbial physiology in response to small signal molecules. *Pseudomonas capeferrum* WCS358 is a plant growth-promoting rhizobacterium whose QS system is regulated by the *N*-acyl homoserine lactones (AHLs) and is composed of the AHL synthase PpuI, the AHL receptor PpuR, the regulator RsaL and a second AHL receptor, PpoR. The aim of this work is the evaluation of the effect of copper on the expression of *ppuI*, *ppoR* and *rsaL*. Transcriptional fusion plasmids based on the pMP220 promoter probe vector were introduced independently into WCS358. The resulting strains were cultivated in solid and liquid medium with and without copper andβ-galactosidase activity was measured. The activities of the *ppuI* and *ppoR* promoters were reduced in half or less in the presence of copper in liquid and solid medium indicating significant differences with the control condition without copper ($p<0.05$, Tukey test). The promoter level of *rsaL* was reduced to a lesser extent than *ppuI* and *ppoR* in the presence of copper in liquid medium but no differences were observedin solid medium ($p > 0.05$, Tukey test). Results presented in this work show that copper modifies the expression of *ppuI*, *ppoR* and *rsaL*, suggesting a concomitant modification of the phenotypes controlled by the QS system in *P. capeferrum* WCS358.

A43
RECEPTORS TNFA-RI AND TNFA-RII IN HOLSTEIN COWS NATURALLY INFECTED BY BOVINE LEUKEMIA VIRUS

Úsuga-Monroy C, Echeverri-Zuluaga JJ, Gónzalez-Herrera LG, Díaz-Castrillón FJ, López-Herrera A.
Biodiversity and Molecular Genetics research group, Universidad Nacional de Colombia, Medellín and Immunovirology Group, Universidad de Antioquia.E-mail: cusugam@unal.edu.co

Bovine leukemia virus (BLV) is a retrovirus that affects the immune system of infected cattle. Its target cell is the B lymphocytes specifically the CD5+ subset. The exposure of B cells to BLV generates the abnormal expression of cytokines and receptors. B cells are increasingly reactive to proliferative signals but they do not have the expected reactivity to the presented antigens. Because of the characteristics of the virus, the antiviral cytokines of the innate response are not efficient to control the viral infection. However, tumor necrosis factor (TNFα) plays an important role in the pathogenesis of BLV infection. The functional activity of TNFα is mediated by two different surface receptors: TNFα-RI and TNFα-RII. TNFα-RI induces cell death through apoptosis, cytokine production and cytotoxicity; whereas TNFα-RII is involved in cell proliferation activities. The objective of this study was to determine the relationship between the expression level of TNFα-RI and TNFα-RII receptors with respect to infection or not by BLV in a population of Holstein cows. A blood sample was taken from 140 Holstein cows that belong to three specialized dairy farms located in the department of Antioquia. DNA and RNA were extracted from the samples. A nested-PCR was performed to amplify a viral env (gp51) gene region and obtain a 444 bp fragment. The level of expression of TNFα-RI and TNFα-RII receptors was determined by qPCR. The association of expression level of the receptors with the presence or absence of BLV was established using a t-test. A higher level of expression of the RI type receptor was found with respect to the RII receptor in all the cows evaluated. The expression level of the RI receptor was higher in the negative cows than in the BLV positive ones ($P=0.0028$). On the other hand, there was no statistical difference between the expression level of the RII receptor between the positive cows and the BLV negative cows ($P=0.560$). According to the results obtained, a higher level of RI receptor expression was obtained in healthy animals compared to infected animals, which may be related to a better ability to produce different cytokines and cytotoxicity, which induces the cell death of infected cells. No statistical difference was found between the level of expression of the RI receptor and RII in the positive cows, it is possible that if the level of expression of both receptors is similar, the cell proliferation or anti-apoptosis routes are promoted, maintaining viral replication.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

MOLECULAR AND CELLULAR BIOLOGY

A44
A PROTEOLYTIC ANTIBODY FRAGMENT THAT INHIBITS JUNÍN VIRUS INTERNALIZATION

Ferrero S, Epting K, Ziegenbein J, Vázquez C A, Payes C, Sawaya M, Cascio D, Daniels-Wells T, Penichet M, Cordo SM, Rodriguez J, Helguera G.
Dept. Química Biológica, (IQUIBICEN-CONICET, FCEyN-UBA) Dept. Chemistry and Biochemistry (UCLA) y Dept. of Surgery, (UCLA) y Laboratorio de Biotecnología Farmacéutica (IBYME-CONICET).E-mail: gustavoh@ibyme.conicet.gov.ar

The Argentine Hemorrhagic Fever (AHF) is a serious endemic disease of the central region of the country, whose etiological agent is the Junín mamarenavirus (JUNV). The current therapy consists of infusions with plasma of convalescent patients, but it exhibits variability and risk of transmission of diseases by transfusion; therefore, new therapies are necessary. The mechanism of entry of JUNV into human cells requires the binding of the viral glycoprotein (GP1) with the transferrin-1 receptor (hTfR1) and occurs via endocytosis. In previous studies we demonstrated that the monoclonal antibody (mAb) ch128.1 against the apical domain of hTfR1, inhibits the entry of JUNV into the cell. However, ch128.1 has human isotype IgG3, so that it can induce cytotoxicity, with risk of affecting healthy tissues. The purpose of this work is to evaluate the feasibility of developing a new monovalent mAb against AHF that lacks Fc domain and inflammatory activity. The Fab fragment of mAb ch128.1 (Fab128.1) was obtained by papain digestion. We evaluated by sandwich-ELISA that the Fab128.1 binds like mAb ch128.1 IgG to the soluble extracellular domain of hTfR1 (sTfR1) obtained commercially from Kerafast or prepared by our group. The JUNV entry block was studied *in vitro* with pseudoviruses decorated with their GP1 / GP2, showing significant effect by Fab128.1 and ch128.1. Similar response was observed on the infection of A549 cells evaluated by plaque formation assay and by qPCR determination of abundance of the RNA of the viral protein Z using an attenuated strain of JUNV. Also, we were able to generate crystals of Fab128.1 and perform structural analysis by X-ray crystallography. This allowed resolving the atomic structure of the complementary determinants regions of the Fab128.1 with a resolution of 2.6Å. Finally, a model of the TfR1-Fab128.1 complex was generated by in silico docking analysis, obtaining a model that shows an overlap of the binding sites of Fab128.1 and the GP1 of a mamarenavirus. These studies confirm that Fab128.1 can bind to the apical domain of hTfR1, block the entry of AHF mamarenavirus *in vitro* like the complete mAb, and to propose a model that explains competition of the mAb for the viral GP1 binding site. Further *in vitro* and *in vivo* studies are needed prove that a monovalent Fab could be an effective and safe therapeutic option against AHF.

A45
GENOTYPING AND STUDY OF ADHERENCE-RELATED GENES OF *Streptococcus uberis* ISOLATES FROM BOVINE MASTITIS

Fessia AS[1], Raspanti CG[1], Odierno LM[1], Dieser SA[1]
[1]Fac. Cs. Exactas, Fco-Qcas y Naturales. Universidad Nacional de Río Cuarto.E-mail: afessia@exa.unrc.edu.ar

Bovine mastitis is a globally widespread infectious disease that is responsible for large economic losses due to lower milk yield and quality. *Streptococcus uberis* is associated with subclinical and clinical intramammary infection (IIM) at any period of lactation. The main source of infection of this microorganism arises from the cow's environment. The pathogenic mechanisms exploited by *S. uberis* are not yet clear, constituting a major obstacle for the development of new control strategies. Bacterial adhesion molecules able to specifically recognize adhesive matrix molecules could ensure the first critical step in tissue colonization. The aim of this research was to determine the presence, conservation and distribution of 6 potential adherence genes and their relationship with diverse molecular types in 34 *S. uberis* strains isolated from bovine mastitis in Argentina. Pulsed-field gel electrophoresis (PFGE) typing with *SmaI* was performed. Dendrograms were generated by using the Dice coefficients and clustering was done by the UPGMA and the cutoff was set at 80 % similarity level. The PCR was standardized for the detection of each gene, *scpA* (SUB_RS05795), *fbp* (SUB_RS05580), *lbp* (SUB_RS00865), *sua* (SUB_RS08150), *lmb* (SUB_RS04460) and *acdA* (SUB_RS03245). The PCR products corresponding to each gene were sequenced in a total of 20 strains of *S. uberis*. Samples of the amplification products were purified and sequenced by the Macrogen service (Republic of Korea). The analysis of the 34 isolates revealed 26 types of PFGE patterns, 21 (80.76%) of which were unique isolates, 1 type containing 2 isolates and 8 subtypes containing 1 isolate per subtype. A high prevalence of *scpA, fbp, lbp, lmb* and *acdA* genes, 100% to 97%, was detected in the 34 *S. uberis* isolates, whereas 27 isolates (79.41%) harbored the *sua* gene. A high degree of similarity in the nucleotide (94%-100%) and amino acid (95%-100%) sequences was determined for analysis of the 6 genes by comparison between 20 field strains and the reference strains. These results demonstrate that, in spite of *S. uberis* clonal diversity, the *sua, scpA, fbp, lmb, and acdA* genes are prevalent and highly conserved, showing their importance to be included in future vaccine studies to prevent *S. uberis* bovine mastitis. To the best of our knowledge, this is the first report that compared these potential adherence genes in sequences from field isolates versus reported GenBank sequences *Streptococcus uberis* 0140J (AM946015.1) and *Streptococcus uberis* NZ01 (CP022435.1).

A46
RELATIVE EXPRESSION OF GENES ASSOCIATED WITH ADHESION TO BOVINE MAMMARY EPITHELIAL CELLS BY *Streptococcus uberis*

Fessia AS[1], Raspanti CG[1], Odierno LM[1], Dieser SA[1]
[1]Fac. Cs. Exactas, Fco-Qcas y Naturales. Universidad Nacional de Río Cuarto.E-mail: afessia@exa.unrc.edu.ar

Streptococcus uberis is one of the most prevalent environmental pathogens responsible for a significant proportion of subclinical and clinical intramammary infections in lactating and no lactating cows. Adherence of *S. uberis* to host epithelial cells has been accepted as an important initial and critical step in the colonization of bovine mammary glands. The aim of this work was to evaluate the relative expression (R) levels of adhesion genes in three *S. uberis* strains in adherence assays. The strains were identified by Mass spectrometer Bruker MALDI-TOF. For the assays the MAC-T bovine mammary epithelial cell line was used. For expression studies total RNA extraction was performed from 3 experimental groups: *S. uberis* cultured alone (C) used as control, *S.uberis* present in the supernatant of co-cultures with MAC-T (T_1), adhered and internalized bacteria (T_2). Total RNA was isolated using Trizol, following manufacturer's instructions. Then DNA was removed using DNase. The reverse transcription of RNA to cDNA was done using Kit High-Capacity cDNA Reverse Transcription, according to manufacturer's instructions. Quantitative real time PCR was carried out using qPCR Kit 2X SuperMix iTaq Universal SRYB Green using the Stratagene MX3000 Pro thermocycler (Applied Biosystem). The stability of the housekeeping gene expression was checked by using BestKeeper Software as described by Pfaffl et al. (2004). The C_T value was used for determine relative expression of *acdA*, *lmb*, *scpA*, *sua*, *fbp* and *lbp* genes, using delta-delta method by Livak et al. (2011). The RC13, RC29 and RC38 strains were able to adhere to MAC-T epithelial cells, showing that the strains exhibit different adhesion capability to MAC-T. The strain RC29 showed the highest adhesion capability. The target genes showed higher values of expression in the T_1 condition compared to the control. While the genes *acdA*, *lmb* and *scpA* showed significant decrease in the T_2 condition compared with control. In the three strains greater expression values were evidenced in the *lmb* and *fbp* genes in the T_1 condition. In general, relative expression of adherence genes in *S. uberis* strains in condition T_2 did not change or decrease compared with control. The results presented in this research evidenced that adherence to MAC-T epithelial cells differ between *S. uberis* strains and that expression of the genes related to adherence is dependent strain. In conclusion, the target genes showed relative expression of different magnitude according to the strain and their interaction with the cells MAC-T.

A47
GENETIC DIVERSITY IN POPULATIONS OF *Nezara viridula* (HEMIPTERA: PENTATOMIDAE) BASED ON MITOCHONDRIAL DNA SEQUENCES

Pérez de Rosas AR, García B A.
INICSA, CONICET y Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba.E-mail: arperez@biomed.fcm.unc.edu.ar

Nezara viridula is a cosmopolitan species that produce important economic damages on different crops. The analysis of genetic variability can provide bases for understanding the dynamic and evolution of natural populations of this insect pest. Previous analyses based on cytochrome oxidase I (COI) gene sequences of *N. viridula* from different localities of Argentina, revealed limited levels of variability. In order to identify potentially useful fragments for the study of the genetic variability in populations of *N. viridula*, mitochondrial sequences corresponding to the subunit 5 of NADH dehydrogenase (ND5) gene and the control region were analyzed in specimens from different Argentinian populations. The comparative analysis of a fragment of 884 bp of the ND5 gene from 23 specimens belonging to three localities, revealed 6 haplotypes determined by 6 variable sites. The total nucleotide diversity was 0.00108 and 0.00184 according to the estimators π and θw, respectively. The mean value of haplotypic diversity (Hd) was 0.680. The moderate levels of genetic differentiation observed among populations and the presence of exclusive haplotypes suggest that gene exchange among populations would not be enough to disperse these haplotypes. On the other hand, a fragment of 1785 bp of the control region of 69 individuals from seven localities was analyzed. The DNA sequence comparison revealed 60 haplotypes determined by 108 variable sites. The total nucleotide diversity was 0.00426 and 0.0126 for π and θw, respectively, and the mean value of Hd was 0.990. Neutrality tests suggested that the majority of the populations would have experienced expansion events. No significant association was detected between geographic distance and genetic differentiation among the sites sampled (Mantel $r = -0.139$, $P = 0.742$). This pattern suggests restricted gene flow among sampling sites, where haplotypes frequencies could drift independently without relation to the geographic distances separating them. It is probable that during the population reductions caused by the insecticides used to control this agricultural pest, the genetic drift would have play a rol in the differentiation and structuring of the populations independent of geographical distance. The results obtained indicated that ND5 and control region sequences would be useful to analyze the dynamic and evolution of *N. viridula* populations.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A48
DAILY VARIATIONS IN THE EXPRESSION OF GENES RELATED TO DISPERSION AND INSECTICIDE RESISTANCE IN *Triatoma infestans*

Stroppa MM, Varela GM, Garcia BA.
INICSA, CONICET y Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina.E-mail: mercedesstroppa@hotmail.com

A variety of daily rhythms in behavioral, physiological, and metabolic processes have been observed in *Triatoma infestans*, the main vector of Chagas disease in the Southern Cone of Latin America. Pyrethroid insecticides have been the major means to control the vector populations and the massive use of chemical insecticides has lead to resistance. The flight dispersal and pyrethroid resistance are important factors for population recovery after insecticide spraying. The *T. infestans* flight muscles have two glycerol-3-phosphate dehydrogenase (GPDH) isoforms with an essential role in the flight metabolism. Isoforms exhibit a temporal expression patterns, sex-differentiated, and changes in relation to temperature and intake. On the other hand, one of the major mechanisms involved in the insecticide resistance is associated with an increase in the expression or activity of oxidative metabolism of the insecticide by cytochromes P450. Previous analyzes of cytochromes P450 and the NADPH cytochrome P450 reductase (CPR) genes expression, in resistant and susceptible populations to deltamethrin, revealed that P450 genes would be involved in the development of resistance to insecticides in *T. infestans*. In order to investigate the presence of rhythms in the expression of genes related with flight metabolism and insecticide resistance, we explored the daily expression profile of GPDH isoforms in flight muscles and the expression profile of the CPR gene and a P450 gene (CYP4EM7) in fat body from adults of *T. infestans* restrained under light/dark cycle (LD), constant light (LL), and constant dark (DD) conditions. In DD, GPDH-1 flight muscles of *T. infestans* showed a rhythmic pattern of transcription synchronous with a rhythmic profile of activity suggesting regulation by the endogenous circadian clock. Otherwise, the GPDH-2 expression analysis showed no regulation by the endogenous clock, but showed that an external factor, such as the dark/light period, was necessary for synchronization of GPDH-2 transcription and activity. In LD condition, the CPR gene expression profile of females showed two significant peaks, conserved in DD and lost in LL. These results suggest that CPR gene expression is under endogenous clock regulation. In males was not observed a rhythmic profile in the expression of the CPR gene. In LD, the expression of CYP4EM7 gene in males and females showed daily significant variations. The expression in females presented a peak at dawn and in males showed two significant peaks, one at dawn and other at sunset. This study would provide potentially useful information to analyze the temporal regulation of important biological processes, such dispersal and insecticide resistance.

A49
INDUCTION OF CELLULAR STRESS IN MURINE MAMMARY ADENOCARCINOMA MEDIATED BY SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPIONS)

Vázquez L, Kun A, Bausero M.
Departamento de Proteínas y Ácidos Nucleicos (IIBCE, Uruguay) y Departamento de Genética (IIBCE, Uruguay).
E-mail: lvazquez@iibce.edu.uy

The use of super paramagnetic iron oxide nanoparticles (SPIONs) is being widely used on cancer therapy research, because they could potentially deliver oncological drugs more efficiently. Although, the possible benefits of the use of SPIONs are considerable, there is a clear need to identify any cellular damage associated with the intracellular presence of these nanoparticles. The study of underlying molecular mechanisms in breast cancer, positions some of the Heat Shock Proteins (Hsps) as possible therapeutic targets. Among them, Hsp27, together with the master regulator of the response Heat Shock Factor 1 (HSF-1), are regulatory points of progression and tumor metastasis. The inclusion of nanotechnology in the design of strategies for targeted therapies provides a wide range of structures that allow direct non-cytotoxic treatment of the tumor in a range of concentrations that depends on each nanoparticle. In this work, we evaluated the induction of HSF-1 and Hsp27 after stimulating with SPIONs by performing indirect immunostaining and western blot studies. We found that treatment with SPIONs produces an increase in cellular stress. The expression of these two markers was differential on the cell lines studied. For instance, 4T1 mouse mammary adenocarcinoma cells showed an increase of 130% of HSF-1 in the nucleus (N) and 240% in the cytoplasm (C); in normal NIH3T3 murine fibroblast cells the increase was 111% N and 106% C; while in murine macrophages J774 was 144% N and 110% C. For the Hsp27 protein, the increase was seen in all the lines and in all the compartments: in 4T1 144% N and 150% C; in NIH3T3 141% N and 134% C; and J774 148% N and 133% C. In conclusion, to the best of our knowledge we were the first ones to describe the potential negative effects of the use of nanoparticles on drug delivery for cancer treatment. Thus, based on the obtained data, we can speculate that SPIONs induction of HSF-1 and Hsp27 may favor the maintenance of an appropriate microenvironment for tumor progression, suggesting a need for improvement on the design of these nanoparticles for the delivery of oncotherapeutic drugs.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

REPRODUCTION I

A50
INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN THE REGULATION OF OXYTOCIN RECEPTOR IN HUMAN PLACENTA

Accialini P[1], Etcheverry T[1], Abán C[2], Negri Malbrán M[3], Leguizamón G[3], Maté S[4], Farina M[1].
[1]Laboratorio de Fisiopatología Placentaria (CEFYBO-CONICET). [2]Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI).[3]Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno (CEMIC). [4]Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP-UNLP).E-mail: paccialini@gmail.com

The onset of labour is a process controlled by fetal, maternal and placental factors. Among the different signals that contribute to labour, oxytocin (OT) is produced not only by the hypothalamus but also by uterus, amnion and placenta. The synthesis of OT and the expression of its receptor (OTR) are increased in placenta at term. Interestingly, changes in OTR expression, rather than the concentration of OT, are considered important for labour onset and progression. Growing evidence indicates that the endocannabinoid system (ECS) is involved in regulating the initiation of labour. Endocannabinoids are a family of lipid-signaling molecules that regulate different physiological processes in the placenta, being Anandamide (AEA) the most relevant one. It exerts its main effects through two cannabinoid receptors: type 1 (CB1) and type 2 (CB2). Besides, some of its effects are achieved through a transient receptor potential vanilloid type 1 (TRPV-1). Anandamide is produced in the placenta and a significant increase in plasma levels are observed in labouring women with respect to patients undergoing cesarean section. Notably, AEA can trigger the release of OT in the rat hypothalamus, suggesting a possible relation between the ECS and OT/OTR signaling. Based on the aforementioned, we hypothesize that the ECS modulates the OT/OTR signaling in human placenta at term. The objectives were: 1) to study the expression of the ECS in human placenta from vaginal delivery (VD) or cesarean section (CS) at term, and 2) to analyze the effect of AEA on OTR expression in placenta at term. To this purpose, placentas from VD or CS were used for RT-qPCR, Western Blot and enzymatic activity. We found a significant decrease ($p<0.05$) in the mRNA and protein levels of FAAH, the enzyme that degrades AEA, in VD placentas. Moreover, we found a significant decrease ($p<0.05$) in its enzymatic activity, in agreement with the increase in AEA levels observed in labouring women. With respect to the endocannabinoids receptors, we found no differences in CB1 protein content between VD and CS placentas, while TRPV-1 protein levels were significantly lower ($p<0.05$) in VD placentas. Besides, explants from CS placentas were cultured in the presence of different concentrations of R-(+)-Methanandamide (Met-AEA), a stable AEA analogous, for 3 or 20 hours. Our preliminary results suggest that CS placentas present a basal lower OTR expression compared to VD placentas. Besides, the culture of CS placentas with Met-AEA for 20 hours produced a significant increase in OTR expression ($p<0.05$). Our findings indicate a differential expression of the ECS between VD and CS placentas, and suggest that EAE may modulate OTR expression in the placenta at term.

A51
INHIBITION OF GABAB SIGNALING IN FEMALE NEONATES ALTERS OVARY GENE EXPRESSION AND DELAYS PUBERTY ONSET

Bizzozzero Hiriart M, Di Giorgio NP, Libertun C and Lux-Lantos V.
Instituto de Biología y Medicina Experimental (IBYME-CONICET, Buenos Aires, Argentina).E-mail: m.bizzozzero@hotmail.com

GABAB antagonist (CGP55845, CGP) administration to neonatal BALB/C mice significantly decreased ARC *Kiss1* expression in both sexes. *Cyp19a1, Esr1/2, Pgr* hypothalamic expression were not affected by this treatment. Nevertheless, ovarian and testicular E2 contents were significantly increased in CGP-treated mice. Here we analyzed the impact of neonatal CGP administration on PND6 ovarian gene expression and ovarian morphology and the effect on body weight (BW), sexual differentiation, puberty onset, and adult hormone levels and estrous cyclicity. Female Balb/c mice were injected with CGP (1 mg/kg, sc) or saline from postnatal day 2 (PND2) to PND6±1, three times/day (8AM, 1PM, 6PM). One set of mice was sacrificed at 3PM (after two injections on the last day). One ovary was collected to determine aromatase (*Cyp19a1),* estrogen receptors (*Esr1/2),* kisspeptin (*Kiss1)* and kisspeptin receptor (*Kiss1r)* gene expression by qPCR; the other ovary was used to analyze ovarian morphology. In the second set of animals, equally treated, BW and anogenital index (AGI=ano-genital distance (AGD)/BW) were evaluated on PND7, PND14 and PND21 and puberty onset was determined by vaginal opening (VO). In adulthood, LH and FSH levels were determined by RIA and estrous cycles were evaluated. CGP increased *Cyp19a1, Esr1 and Esr2* expression in neonatal ovaries(*Cyp19a1*: CTRL:0.97±0.32 vs CGP:2.5±0.55: p<0,04;*Esr1*:CTRL:0.85±0.30 vs CGP:3.80±0.54: p<0,001;*Esr2:* CTRL:1.11±0.39 vs CGP:6.99±1.65: p<0,003). Neither *Kiss1* nor *Kiss1r* ovarian expression was altered by CGP (ns). PND6 ovaries from CGP-treated mice showed an increase in primordial follicles ($p<0.025$). CGP decreased PND7 BW, increased PND7 and PND21 AGI and delayed puberty onset (VO/BW). Adult FSH was decreased in CGP-treated mice, nevertheless estrous cycles were not affected. Our data clearly show that lack of GABAB signaling stimulates the ovarian estrogenic system and prevents the decrease in the neonatal primordial follicle reserve. CGP55845 also impacts BW, sexual differentiation, puberty onset and adult FSH levels, while not altering estrous cycles. Therefore, transient neonatal GABAB signaling impairment induces long-lasting effects in the reproductive axis of female mice. *Supported by CONICET, ANPCYT, UBA, Fundación René Barón, Fundación Williams.*

BIOCELL 43 (suppl. 3), 2019 ABSTRACTS
ISSN 0327- 9545 A1 – A104
ISSN 1667-5746 (online version)

A52

Pomacea canaliculata EGGS ARE DEFENDED AGAINST FROG PREDATION BY ALTERING THEIR SMALL INTESTINE MORPHOPHYSIOLOGY

Brola TR[1], Dreon MS[1], Fernández PE[2] and Heras H[1].
[1]Instituto de Investigaciones Bioquímicas de La Plata "Profesor Doctor Rodolfo R. Brenner" (INIBIOLP), CONICET-UNLP, La Plata, Argentina. [2]Instituto de Patología B. Epstein, Cátedra de Patología General, Facultad de Cs. Veterinarias, UNLP, Argentina.
E-mail: tabatabrola@gmail.com

The invasive freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae), native from South America, has been introduced in different parts of the world causing serious ecologic, economic and human health problems. Though these snails lay their eggs above water, exposing them to terrestrial predators, they have virtually no reported predators. We have recently shown that the perivitelline fluid (PVF) that surrounds the embryo, besides nourishes them, contains a cocktail of lectins, neurotoxins, protease inhibitors and antinutritive proteins involved in a unique defence system against predation. This defence system is similar to the one thoroughly studied for plants seeds against herbivorous animals but has not been previously described in animals other than *Pomacea* genus. Previously we demonstrated the PVF proteins, hereafter perivitellins, resist extreme pH conditions and digestive protease degradation, withstanding the passage through the gastrointestinal tract of rodents and simultaneously affecting gut morpho-physiology. These effects have only been reported in mammal models and whether these perivitellins have nutritional implicances in other taxa of potential predators was unknown. In this work, we evaluate the antinutritive and antidigestive properties of *P. canaliculata* perivitellins in *Lithobathescatesbeianus* as an amphibian model of a potential predator. We orally gavaged PVF to frogs at 24h and 48h, before animals were euthanized and the anterior part of the small intestine analysed by conventional histology, immunohistochemistry and lectin-histochemistry (LHC). Macrophages were determined using CD68 monoclonal antibodies. Marked changes in villi morphology were observed as well as a decrease in the small intestaine absorptive surface area when compared to control group. A significant increase of the connective tissue and the number of inflammatory cells, especially eosinophils and macrophages, were also observed. The gut epithelium of treated frogs binds the snail toxic lectin Perivitellin-1 (PcPV2) and LHC showed alterations in enterocytes´ glycocalyx glycosylation pattern. As a whole these results indicate that *P. canaliculata* PVF has a strong effect on frog digestive system by altering the normal morphophysiology of the digestive system, an effective defence for deterring predators from consuming the egg.

A53

CYCLOOXIGENASE-2 MEDIATES ANANDAMIDE EFFECT DURING IMPLANTATION IN THE RAT

Cañumil VA[1], Beltrame JS[1], Sordelli MS[1], Franchi AM[1], Meresman G[2], Ribeiro ML[1].
[1]CEFYBO (CONICET – UBA), [2]IBYME (CONICET).E-mail: canumilvanesa@gmail.com

Anandamide is a lipid mediator from the group of endocannabinoids that participates in embryo implantation. Anandamide tone is regulated by its degrading enzyme, fatty acid amide hydrolase (FAAH) and dysregulations in anandamide production are correlated with implantation failure and recurrent abortion. Also, cyclooxigenase-2 derived prostaglandins play a crucial role at implantation sites. During early pregnancythe endometrial stroma is mainly formed by fibroblasts, and later when embryo invasion begins, these fibroblasts develop into decidual cells. Yet, the role of stromal fibroblasts during early gestation remains unexplored. Also, it has been described that anandamide and prostaglandins regulate fibroblast behavior in many biological systems. Therefore, the aim of the present work was to investigate the crosstalk between anandamide and cyclooxigenase-2 in stromal fibroblasts during the implantation process.We adopted experimental designs *in vitro* and *in vivo*. First, Wistar females were sacrificed in day 5 of gestation, uterine horns were excised and incubated for 1 h with anandamide 1 nM alone or in the presence of indomethacin 1 µM (a non-selective cyclooxigenase-1 and 2 inhibitor) or meloxicam 1 µM (a highly selective cyclooxigenase-2 inhibitor). Day 5 pregnant rat uterus (day of implantation) is principally composed by stromal fibroblasts. We determined the production of prostaglandins by radioimmunoassay. For the *in vivo* approach, another group of rats received an intra-uterine injection of URB-597 (a FAAH selective inhibitor, 2 µl, 1 mM) in the left horn in day 5 of gestation. The right horn was injected with vehicle and considered as control. Parameters related to implantation events and cyclooxigenase-2 protein level (western blot) were studied in day 8 of pregnancy. We observed that incubation with anandamide increased the production of prostaglandins E2 (11.1 ± 0.8 vs 17.6 ± 0.6 pg/mg ph, $p<0.05$) and F2alpha (70.3 ± 2.0 vs 104.4 ± 4.6 pg/mg ph, $p<0.05$). The pre-incubation of uterine strips with indomethacin or meloxicam reversed anandamide stimulation, suggesting that cyclooxigenase-2 mediated anandamide effect. Dysregulation of anandamide tone *in vivo* due to treatment with URB-597 increased the percentage of embryonic resorptions (1.0 ± 0.1% vs 52.0 ± 10.0%, $p<0.05$) in 100% of females without modifying the number of implantation sites. Also, in 83% of cases we observed aberrant embryo spacing. Finally, cyclooxigenase-2 protein level was augmented in resorbed implantation sites compared to control. Altogether, these results indicate that anandamide might regulate processes that depend on stromal fibroblasts by a mechanism that involves cyclooxigenase-2.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A54
REPRODUCTIVE STRATEGIES OF GASTROPODS. BIOCHEMICAL COMPOSITION OF THE EGGS OF THE FRESHWATER SNAIL *Pomacea diffusa*

Chiumiento IR, Brola TR, Dreon MS, Heras H.
Instituto de Investigaciones Bioquímicas de La Plata "Profesor Doctor Rodolfo R. Brenner"(INIBIOLP), CONICET-UNLP, La Plata, Argentina.E-mail: igchiumiento@gmail.com

The freshwater snails of *Pomacea* genus lay their egg masses above water level on emergent substrates. This is an unusual reproductive strategy for an aquatic animal, exposing egg to stressful environmental conditions as well as a wide range of terrestrial predators. In spite of this, these eggs have a high hatching rate and have no described predators in their native distribution range. It is remarkable that some species, such as *P. canaliculata* and *P. maculate* (canaliculata clade) have become important invasive species out of their native distribution ranges while others such as *P. scalaris* and *P. diffusa* (bridgessi clade), do not have this invasiveness. In previous works, our research group has stablished the role of some egg proteins (hereafter perivitellins) in the adaptation to harsh developmental conditions and in the egg defense against predation. We found interesting structural and functional differences between invasive and non-invasive species on their principal perivitellins. Our general objective is to shed light on the comparative aspects and adaptation mechanism among *Pomacea* species associated with their invasiveness and geographical distribution and on the role perivitellins play on aerial egg adaptations. The aim of this work was to characterize the biochemical composition of the *P. diffusa* eggs, a non-invasive *Pomacea* species belonging to the bridgessi clade and compare it with those previously obtained for other species, focusing on their proteins. The egg masses biochemical composition was dominated, as in the previously studied species by polysaccharides (0.12 g/g) and proteins (4.29 mg/g) being lipids less represented (0.28mg/g). Egg masses electrolyte concentration was dominated by chloride (0.15 mg/g), followed by sodium, potassium, calcium and magnesium with 0.06, 0.025, 0.024 and 0.015 mg/g respectively. Regarding the egg protein composition, it is dominated by a carotenoprotein (>50% of total soluble protein) with a high hydration density (1.26 g/mL) and a molecular mass of ca. 420 KDa, composed by several ~30 KDa subunits. This protein composition dominated by a single high MW oligomeric protein was previously observed in the other *Pomacea* species. As a whole, these results represent the starting point for future studies on the biochemical defenses of *P. diffusa* eggs aiming to better understand the reproductive biology of the genus and its associated ecological and evolutionary implications.

A55
DIMETHYLTHIOUREA MODIFIES GSH CONCENTRATION AND MALE PRONUCLEAR FORMATION IN PIGS OOCYTES

Ghersa J [2], Lorenzo MS [1,2], Luchetti CG [1,2], Teplitz GM [1,2], Cruzans PR [1,2], Lombardo DM [2].
[1]CONICET. [2]Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Instituto de Investigación y Tecnología en Reproducción Animal (INITRA). Cátedra de Histología y Embriología. Chorroarin 280, CABA, Argentina.
E-mail: soledadlorenzo@fvet.uba.ar

Successful *in vitro* embryo production is determined amongst other factors by sustaining a balanced redox state during oocyte maturation. *In vitro* matured oocytes are subject to higher oxygen levels (20%) than those in vivo (7%). Manipulation of oocytes also contributes to high levels of reactive oxygen species (ROS) production and resulting oxidative stress. It is known that sperm chromatin decondensation and later male pronuclear (MPN) formation is related to oxidative stress and glutathione (GSH) levels in oocytes during *in vitro* maturation (IVM). We previously reported that the antioxidant dimethylthiourea (DMTU) reduces ROS level in oocytes. This study aimed to evaluate the effects of different concentrations of DMTU on matured oocyte GSH concentrations and MPN formation. We obtained pig COC through follicular aspiration from slaughterhouse ovaries. Three treatments were applied during aspiration and further search: PBS + 10% porcine follicular fluid (control group); or supplemented with 1mM of DMTU (1 mM treatment); or 10 mM of DMTU (10 mM treatment). Oocytes were matured *in vitro* in wells containing supplemented Medium 199, under 5% of CO_2 in humidified air at 39ºC, during 44 h. COC were denuded with hyaluronidase and oocytes (30 per tube, 8 tubes per treatment) were homogenized in PBS on ice and centrifuged at 4500 x g for 10 minutes. Total GSH was measured through colorimetric assay adapted to 96 well microplates, and it was relativized to protein level (measured by Bradford technique). IVF was performed with fresh boar semen ($1x10^6$ sperm/mL) in modified Medium 199, under 5% of CO_2 in humidified air at 39°C, during 6 h. MPN formation was evaluated through Hoechst stain after 20 h of IVF (n control= 77; 1 mM treatment=96, and 10 mM treatment=87). There was a significant decrease in GSH levels in the 10 mM treatment in comparison with control and 1 mM treatment ($p<0.05$). MPN formation was significantly less in the 10 mM treatment than those in the 1 mM treatment and control ($p<0.05$). We concluded that the addition of 10 mM of DMTU during aspiration and searching does not represent a benefit as it reduces MPN formation and GSH concentrations. We did not find differences between control and the 1 mM treatment. However, considering our previous reports, the use of 1 mM of DMTU needs to be further analyzed to determine its efects on embryo development.

A56
EFFECTS OF A HIGH-FAT DIET ON MALE FERTILITY IN HIGH FERTILITY PERFORMANCE MICE

Rainero Caceres TS, Gomez Elias MD, Giaccagli M, Dalton N, De Siervi A, Cuasnicu PS, Cohen DJ, Da Ros VG.
Instituto de Biología y Medicina Experimental (IBYME-CONICET).E-mail: tomas.rainero.c@gmail.com

The metabolic syndrome (MeS) is defined as an increase in 3 of the following factors: visceral adiposity, triglycerides, cholesterol, fasting glucose and blood pressure. Considering the worldwide rise in the prevalence of this syndrome during the last years, the aim of this study was to evaluate MeS effects on male fertility in mice with high reproductive performance. For this purpose, three-week-old hybrid (C57BL/6xBALB/c) F1 male mice received *ad libitum* a control diet (CD, 6% fat) or a high-fat diet (HFD, 30% fat) for 19 weeks. HFD mice ingested a higher amount of fat than CD mice (0.97±0.04 vs 0.25±0.01 g of fat/day, n=9, p<0.01). However, as HFD males consumed less total food than CD males (3.2±0.1 vs 4.2±0.1 g of food/day, n=9, p<0.01), these animals daily consumed only 12% more calories than CD animals (14.9±0.6 vs 13.1±0.4 Kcal/day, n=9, p<0.05). Thus, these results indicated that HFD animals received a poor-quality diet. Starting from the 13th week of treatment, HFD mice gained more weight compared to CD mice (at week 19: 36.4 vs 31.7 g; n=9, p<0.001). While serum levels of triglycerides were similar in both groups, there was a significant increase in cholesterol (at week 19: 1.3±0.12 vs 0.7±0.06g/l, n=9, p<0.001) and fasting glucose (at week 18: 167.8±9.4 vs 134.0±9.5 mg/dl, n=9, p<0.05) in HFD mice, consistent with the acquisition of MeS. Moreover, glucose intolerance after glucose administration (ip, 2 g/kg of body weight) was evident in HFD mice compared to CD mice at week 18. In order to determine the impact of this syndrome on male fertility, males were caged for one night with females in proestrous stage and the next morning the eggs were collected and cultured in KSOM medium. There was no significant difference between both groups in the fertilization rate (as percentage of 2-cell embryos) or in the percentage of embryos that developed to blastocyst 4 days later. While the male reproductive tracts were normal under macroscopic observation in both groups, there was a higher amount of gonadal fat in HFD mice compared to controls (at week 18: 1.32±0.17 vs 0.59±0.04 g, n=4, p<0.01), which could potentially impair testicular and/or epididymal function. Based on this, we next performed *in vitro* studies, as a more restricted condition to unveil possible sperm defects. Whereas there was no difference in sperm viability, motility or acrosome reaction between groups, sperm count was lower in HFD compared to controls (at week 18: 72.8±8.8 vs 99.8±8.6 x10^6cells/ml, n=9, p<0,05). Finally, *in vitro* fertilization assays employing cumulus-intact oocytes showed no differences in the fertilization rate or *in vitro* embryo development between groups. In summary, although HFD altered some sperm function parameters, it did not impair male fertility in high reproductive performance mice. The effect of HFD in animals with a lower reproductive performance is under investigation.

ONCOLOGY

A57
A NOVEL NANOCARRIER FOR DOCETAXEL

Albano JMR, Nunes de Morais Ribeiro L, Muniz Couto V, Barbosa Messias M, Rodrigues da Silva GH, Breitkreitz MC, de Paula E, Pickholz M.
Departamento de Física, FCEN, Universidad de Buenos Aires, Argentina. Instituto de Física de Buenos Aires (IFIBA), CONICET-Universidad de Buenos Aires, Argentina. Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, Universidade Estadual de Campinas – UNICAMP, Campinas - São Paulo, Brasil. Institute of Chemistry, University of Campinas – UNICAMP. Campinas, SP, Brazil. E-mail: jmra88@gmail.com

Notwithstanding the advances in diagnosis and treatment, cancer still has a high impact on public health. Encapsulation of ant-cancer drugs in nanoparticles has shown improvement of the therapeutic efficiency and safety of them. To tackle with this, we designed a nanocarrier for the encapsulation of the anti-cancer drug Docetaxel. The nanocarrier was developed aiming to minimize the proportion of excipients in the optimized formulation and it was based on solid lipid nanoparticles. Through a factorial design, experiments were performed to study the effects of the excipients on the possible formulations and improve their ratios. DSC, X-ray diffraction, FTIR-AR and Molecular Dynamics Simulation studies were carried on to characterize the obtained formulations. Dynamic light scattering and nanotracking analysis revealed the distribution size of the particles, compatible with viable nanomedical applications. Almost complete loading of the drug into the nanocarrier was achieved, as explored with encapsulation and release assays. The resulting formulations could be suitable in the treatment of solid tumor cancers, decreasing the side effects commonly associated with the administration of Docetaxel.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A58
ROLE OF WNT/BETA-CATENIN SIGNALING IN OVARIAN TUMOUR GROWTH AND ANGIOGENESIS. A CROSSTALK WITH NOTCH SYSTEM

Bocchicchio S, Tesone M, Irusta G.
Laboratorio de Fisiología y Biología Tumoral del Ovario, Instituto de Biología y Medicina Experimental (IByME-CONICET).
E-mail: sebastianbocchicchio@gmail.com

Wnt/β-catenin and Notch are highly conserved pathways that regulate a diversity of cell processes, including proliferation, apoptosis and differentiation. We analyzed the role of both systems in ovarian cancer using specific inhibitors. For this purpose we performed three *in vivo* experiments. A human ovarian adenocarcinoma cell line (IGROV-1) was subcutaneously injected in 6-8 weeks-old female nude mice. Once the tumours were palpable, we injected the inhibitors: the first and second experiments were carried out using Wnt/β-catenin inhibitors (XAV939: 2.5, 5 mg/kg; ICG-001: 5 and10 mg/kg). The third experiment was a combination of ICG-001 (5 mg/kg) and DAPT (5 mg/kg), a Notch inhibitor. Mice were injected every two days three times and they were euthanized 3 days after the last injection. Our results showed a significant decrease in tumour size when mice were treated either with XAV939 or with ICG-001. When compared with tumours from non-treated animals, both experiments showed a significant decrease in cell proliferation (KI67) and a decrease in the endothelial and periendothelial cell area stained with CD31 and α-Smooth-muscle-actin, respectively. When mice were treated with XAV939, a significant decrease in VEGF levels and Angiopoietin 1/2 was observed. Regarding the experiment with the combination of inhibitors, there was a significant decrease in tumour size and a decline in tumour cell proliferation (KI67). Both inhibitors administered simultaneously produced a decrease in cell proliferation at the same extent as individually administrated. In conclusion, we demonstrate a clear involvement of Wnt/β-catenin in ovarian tumour growth and angiognesis. We suggest an interaction of this pathway with Notch system.

A59
IN VITRO ANTI-CANCER ACTIVITY OF FUNGAL PROTEINS ADSORBED ON SILICA NANOPARTICLES

Fusco M[1], Rocha G[1], DesimoneM[2], Diaz ME[1], Mitarotonda R[1,2].
[1]Departamento de Ciencias Básicas e INEDES-CONICET, Universidad Nacional de Luján y [2]FFyB, Catedra de Química Analítica e Instrumental, IQUIMEFA-CONICET, UBA.E-mail: rominamitarotonda@hotmail.com

The anticancer treatments that are currently used have a high toxicity due to the lack of specificity, since they affect tumor cells and healthy cells. Therefore, research in this field focuses on the discovery of new drugs obtained from natural sources that selectively kill cancer cell and new tools like nanoparticles that could increase stability and could be used as delivery system to targeted organs or cells. The aim of this work was to evaluate the cytotoxic capacity on cancer cells of fungal proteins adsorbed on silica nanoparticles (SiO2NPs).In order to achieve this goal, an extract rich in proteins of the edible mushroom *Agaricus bisporus* was obtained by extraction with 5% acetic acid followed by ethanolic precipitation. The proteins presents in the extract were separated by size-exclusion liquid chromatography (SEC-FPLC). Besides, negative nanoparticles were synthesized by the Stöber method and characterized by TEM, DLS, and potential Z. The SiO2NPs obtained were homogeneous population and had a size of 80.0± 5.7 nm. The protein fraction (PF) was incubated with the nanoparticles at 500 rpm and 4 ° C for 6 h to allow the adsorption of the proteins on the SiO2NPs. After incubation, it was obtained that the adsorption capacity of proteins was 5-15μg/0.05 mg NPs (determined by SDS-PAGE and Bradford method). The adsorption of the proteins on the nanoparticles (SiO2NPs-PF) remained stable during at least 20 days. The cytotoxic capacity of the proteins and SiO2NPs-PF was evaluated on MCF-7 cells and PBMC human cells by a colorimetric test using 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide. In addition, the cytokines and growth factors levels were evaluated by ELISA (IL-1β, IL-6, TGF-β). The results showed that one PF had antitumor activity with high cytotoxicity against MCF-7 cells (82%). We obtained that the PF was nontoxic on this cells (7.3%) and cytokine levels did not present significant differences with control. Moreover, SiO2NPs-PF had a higher antitumor action on MCF-7 cells (94%) and did not affect human red blood cells (0.6-2.1% hemolysis) or PBMC cells. These results allow us to continue with the studies in the search for new chemotherapeutic treatments and tools that improve the specificity and bioavailability for the fight against cancer.

A60
GANGLIOSIDE MICELLES LOADED WITH ONCOLOGICAL DRUGS CAN BE COATED WITH SPECIFIC ANTIBODIES AS STRATEGY TO IMPROVE TARGETING

Garro AG[1], Alasino RV[1,2], Leonhard V[1,2], Heredia V[1] and Beltramo DM[1,2,3].
1-Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR-Córdoba); 2-(CONICET); 3-Cátedra de Biotecnología (FCQs-UCC, Córdoba).E-mail: arielgarro10@gmail.com

Efficient and site-specific delivery of therapeutic drugs is a critical challenge in clinical treatment of cancer. A common strategy to achieve higher specificity is modifying surface nanocarriers with specific recognition ligands. Recently, we demonstrated that GM1 micelles carry paclitaxel and doxorubicin with high efficiency and that spontaneously bind to albumin forming GM1-drug-albumin complex. In this work, we show that these micelles, under specific conditions, can be loaded with antibodies to form the GM1-drug-

IgG complex that could enhance specific site accumulation. We evaluate the influence of temperature and pH to obtain stable GM1-IgG complexes and also if the presence of antibodies affects the micellar structure and its capacity to load medications. Under optimal conditions, it is possible to obtain stable GM1-IgG complex until ratios 4/1 (w/w). The DLS and TEM results shows that GM1-IgG complexes have sizes significantly higher than those of GM1 micelles; which is directly related to the amount of IgG loaded. This GM1-IgG complex retains the ability to encapsulate drugs; however, an adequate sequence must be followed during the preparation in order to obtain efficient GM1-drug-IgG ternary complexes. We also showed that the antibodies of the GM1-IgG complex are located with the Fab region in the external domain of the micelles, maintaining their antigenic recognition properties against soluble and cellular antigens. On the other hand, the IgG of the complex is not displaced from the micelles in the presence of albumin.

A61
TREATMENT WITH GM1-PTX MICELLES LEADS TO A POTENT INHIBITION OF METASTASIS IN A MURINE MODEL OF MAMMARY GLAND CANCER

LeonhardV[ab], AlasinoRV[ab], PasqualiniME[bc], CremonezziDC[d], García NH[bc], Beltramo DM[abe].
[a]Centro de Excelencia en Productos y Procesos de Córdoba (CEPROCOR); [b]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); [c]Instituto de Investigaciones en Ciencias de la Salud- FCM (INICSA-CONICET); [d]Cátedra de Patología - Hospital Nacional de Clínicas - Universidad Nacional de Córdoba - Argentina; [e]Facultad de Ciencias Químicas - Universidad Católica de Córdoba – Argentina.E-mail: vickyleonhard@hotmail.com

The formulation of GM1-Ptx micelles was developed with the objective of reducing the toxicity related to the use of Taxol, Cremophor-based paclitaxel and to achieve an easy preparation methodology with greater stability than that of Abraxane, Albumin-based paclitaxel. Acute toxicity of the micellar formulation was evaluated after intravenous administration in mice. The maximum tolerated dose (MTD) obtained for the GM1-Ptx formulation was 55 mg/kg while the lethal dose 50 % (LD50) was 70 mg/kg, this value being higher than that of the commercial formulations Taxol and Abraxane, with LD50 of 30 and 45 mg/kg, respectively. Antitumor activity, mortality and incidence of metastasis were studied in a murine model of mammary gland cancer. The micellar formulation was administered i.v. at different doses for 9 weeks of treatment in comparison with the empty GM1 micelles and saline. After treatment, a complete histological examination of mice and the quantification of various biochemical markers were performed. The treatment of cancerous mice with doses of 30 mg/kg per week achieved greater tumor regression, longer survival and lower incidence of metastasis. When this dose is reached with two weekly administrations, the best results were observed with the least presence of pulmonary micrometastases.

A62
CLEAR CELLS RENAL CARCINOMA: THE ROLE OF PROTEIN BMP7 AND ITS DIFFERENTIAL EXPRESSION

Olea G[1], Melana Colavita JC[1], Barnes T[1], Rodríguez JP[1], Delfino MI[2], Lombardo D[3], Aguirre MV[1].
[1] Universidad Nacional del Nordeste. Facultad de Medicina. Laboratorio de Investigaciones Bioquímicas (LIBIM) IQUIBA-NEA UNNE- CONICET. [2] Servicio Anatomía Patológica Hospital "Juan R Vidal" Corrientes. [3] Universidad de Buenos Aires. Facultad de Cs Veterinarias. Instituto de Investigación y Tecnología en Reproducción Animal (INITRA). Cátedra de Histología y Embriología.

The complex biology of the ccRCC, added to its unpredictable clinical behavior, highlights the need to determine other biomarkers to evaluate neoplastic progression, as well as to generate new therapeutic alternatives taking into accounts the intratumoral heterogeneity. Bone morphogenetic proteins (BMPs) regulate diverse cellular processes, such as proliferation, differentiation, and apoptosis. Among the BMPs isoforms, BMP 7 has been studied in several cancers, but thus far contradictory results have been obtained. This study aimed to clarify the role and significance of BMP7 expression in ccRCC. We performed a study of BMP 7 expression in surgical renal ccRCC samples and in the human renal carcinoma cell line Caki-2 by immunohistochemistry and immunocytochemistry, respectively. Proliferation was evaluated with Ki-67. Tumor samples were collected from patients diagnosed with ccRCC who underwent radical or partial nephrectomy. The clinical-pathological evaluation was performed at the Pathological Anatomy Service of the Hospital J.R. Vidal (Corrientes). Sections of 4 µm of ccRCC tumors and normal adjacent kidney tissue were included in paraffin and placed on silanized slides. Additionally, Caki-2 cells (ATCC® HTB-47™) were cultured on cover glasses, and they were fixed with methanol (99,8 %) for 1 min and were kept until use for BMP7 detection by immunocytochemistry. The conditions for antigen retrieval and the incubation with the BMP-7 antibody (Santa Cruz Biotechnology, 1:200) were adjusted. BMP7 was differentially expressed between the core and periphery regions. Immunoreactions occurred mainly at the perinuclear level, and it was related to the pathological stage and nuclear grade. BMP7 expression was lower in controls. This study reveals that BMP7 is overexpressed in ccRCC samples according to the degree of invasiveness and pathological stages compared to control distal samples. BMP7 expression in CAKI-2 confims a perinuclear pattern similar to cells ccRCC. This preliminary data aims us to design future studies through qPCR and other molecular biology techniques for comparing BMP7 expression with other potential biomarkers such as the pluripotential protein OCT4, in search of tumor stem cells identification.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A63
RELATIONSHIP BETWEEN HYPOXIA AND PROTEOLYTIC ACTIVITY IN TWO CLEAR CELL RENAL CARCINOMA CELL LINES: CAKI-1 AND CAKI-2 CELLS

Palomar LS, Melana-Colvita JP, Barnes TE, Olea G, Aguirre MV, Gay CC, Rodríguez JP.
Laboratorio de Investigaciones Bioquímicas de la Facultad de Medicina (LIBIM-UNNE).E-mail: Lucaspalomar@yahoo.com.ar

Metastasis is the main mortality cause in cancer patients (90%) and particularly this is important in clear cell renal carcinoma (CCRC). Likewise, extracellular proteolysis is one of the main mechanisms that allow the degradation and remodeling of the extracellular matrix and the basement membrane, thus favoring proliferation and tumor metastasis. Recent studies have established a link between this factor and intratumoral hypoxia conditions. Proteolytic enzymes, such as metalloproteinases (MMPs), belonging to a broad family of Zn^{2+}-dependent enzymatic proteins, regulate this microenvironment by controlling angiogenesis and tissue remodeling. Up to date, these studies have not yet been addressed on CCRC. For that reason, in the present work the characterization of the extracellular proteolytic activity was initiated, using the zymography technique for its detection, in "*in vitro*" cultures of two CCRC cell lines: Caki-1 and Caki-2 cells. Both cell lines were exposed to chemical hypoxia, generated by $CoCl_2$ addition (0-300 □M) for 24 h. Then, cellular supernatants were collected for protein and zymographic profile analysis. Additionally, the enzyme activity inhibition assay was performed, using EDTA (50 mM). The preliminary results found, show differential proteolytic activity between both cell lines, being higher in Caki-1 cells, as well as variations among the different treatments. Also, the inhibition experiments demonstrate that the proteolytic activity can be attributed to metallo-dependent enzymes. Currently, using immunochemical assays, we are looking for the action of different MMP isoforms in the process.

A64
E-CADHERIN AND β-CATENIN EXPRESSION IN CUTANEOUS SQUAMOUS CELL CARCINOMAS OF DOGS

*Sanz Ressel BL[a, b, *], Massone AR [c], Barbeito CG[a,b].*
*[a] Laboratorio de Histología y Embriología Descriptiva, Experimental y Comparada (LHYEDEC), Facultad de Ciencias Veterinarias (FCV), Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina.[b] FCV, CONICET, UNLP, La Plata, Buenos Aires, Argentina.[c] Laboratorio de Patología Especial Veterinaria Dr. Bernardo Epstein, FCV, UNLP, La Plata, Buenos Aires, Argentina.*E-mail: lsanzressel@fcv.unlp.edu.ar*

Cutaneous squamous cell carcinoma (CSCC) represents one of the most common malignant neoplasms of the dog skin. Research aimed at clarifying how the deregulated activity of different molecules contributes to CSCC progression can help to identify molecular suitable targets for the development of novel therapies. In this regard, different studies have demonstrated that the downregulation of the expression of E-cadherin and β-catenin contribute to the invasive and metastatic behavior of the cancer cells in human CSCCs. The propose of this study was to evaluate the immunohistochemical expression pattern of E-cadherin and β-catenin on sections of two tissue microarrays constructed from 17 samples of normal epidermis, 12 samples of preneoplastic epidermis and 150 samples of CCEC of dogs, to elucidate the involvement of E-cadherin-β-catenin adhesion system in the progression of canine CSCC. In the normal canine epidermis, β-catenin and E-cadherin were expressed in the membrane of the epidermal cells of the basal, spinous and granular stratum. In the preneoplastic epidermis, the membrane immunoreactivity of β-catenin and E-cadherin was conserved, whereas a significant reduction or loss of the membrane expression of both proteins was observed in CSCCs. These findings show that the dysregulated activity of the β-catenin and E-cadherin may play an important role in the acquisition of malignant phenotype of the keratinocytes and tumor progression during the canine epidermal carcinogenesis process.

A65
THREE-DIMENSIONAL CELL CULTURES MIMIC FIBROBLAST RELEVANCE ON MELANOMA MICROENVIRONMENT AND PHOTODYNAMIC THERAPY IMPACT

Vera RE[1], Morales Vazconsuelo AB[1], Fernández Zapico ME[2], Rivarola VA[1], Rumie Vittar NB[1].
[1]Universidad Nacional de Río Cuarto (UNRC). [2]Mayo Clinic.E-mail rvera@exa.unrc.edu.ar

Melanoma is a neoplasm derived from genetic alterations in melanocytes of the skin. The progression of this disease depends on physical and paracrine interactions among the diverse populations that coexist inside the tumor. From this idea comes the concept of tumor microenvironment (TME), as a complex network of interactions that underlie malignant progression. Carcinoma Associated Fibroblasts (CAF) represent a tumor population that enhance malignancy by transcription factors, among which GLI is important to consider. Preclinical studies on 2D cell cultures are valuable to screen antitumoral compounds or active molecular pathways in melanoma. However, differential results between in vitro and in vivo assays have been observed. This fact indicates the importance to consider the structure and composition of TME. Firstly, to evaluate the influence of CAF expressing GLI on melanoma biology, we achieved a 2D migration assay of melanoma cells (SKMel-2) stimulated by conditional media from CAF (+GLI). The results denoted that GLI activity increased migration around 40 % respect to control media ($p< 0.001$). Secondly to mimic TME complexity, we implemented a 3D heterotypic co-culture of melanoma cells with CAF (F88.2) or tumor cells alone (homotypic spheroids). Microscopy analysis showed that CAF presence modifies cancer cells morphology. Additionally, the cellular migration from spheroids

was higher when fibroblasts were present ($p< 0.05$). Finally, we decided to subjected spheroids to photodynamic therapy (PDT). As a result, cellular migration was delayed even for 72 h ($p< 0.05$). Notably, viability was reduced around 10 % in heterotypic spheroids compared to 25 % ($p< 0.0001$) in homotypic ones. In conclusion, 3D platform is a suitable method to evaluate the influence of CAF in melanoma where GLI influenced malignant behavior. Furthermore, PDT on 3D system was able to delay melanoma migration. These findings enrich our view on phototherapy to accomplish best outcomes.

ANIMAL AND PLANT BIOLOGY AND BIOTECHNOLOGY II

A66
BEHAVIOR OF DIFFERENT CELL LINES IN CONTACT WITH SURFACES BASED ON POLY-*N*-ISOPROPYLACRYLAMIDE

Capella V[1], Rivero RE[2], Liaudat AC[1],Roma DA[3], Mañas F[3], Barbero CA[2], Bosch P[1], Rivarola CR[2], Rodríguez N[1].
[1]Department of Molecular Biology. National University of Río Cuarto-CONICET. Argentina. [2]Department of Chemistry. National University of Río Cuarto-CONICET, Argentina. [3]Department of Animal Clinic. National University of Río Cuarto, Argentina.
E-mail: virginiacapella@gmail.com

In the field of tissue engineering, great advances have been generated in order to create biomaterials that function as scaffolds for cell growth. Several hydrogels simulate the mechanical propertiesofextracellular matrix (ECM), due to its innate similarity in structure and composition, providing a platform that mimic the native cellular milieus and allow a correct growth of several cells. Hydrogel based on *N*-isopropylacrylamide (PNIPAM) is one of the most studied materials in biomedical field; however more extensive biocompatibility and cellular interactions studies with different cell lines are needed to corroborate its biocompatibility. Therefore, the aim of this study isevaluate the biocompatibility of PNIPAM through cytotoxicity, genotoxicity, proliferation and cell adhesion tests inmurine pre-adipose cells (3T3-L1), human embryonic kidney cells (HEK293) and human carcinoma-derived cells (A549). MTT and neutral red uptake assays shown noncytotoxic effect of PNIPAM in any of the studied cell lines. Genotoxicity was evaluated by Comet Assay, where DNA damages were not detected. [^{3}H]-thymidine staining allowed corroborating that cell cycle had progressed normally, allowing a correct proliferation. Adopted morphologies for each cell line over PNIPAM indicate that the surfaces favor the cell attachment during five days culture. The good biocompatibility of PNIPAM surface makes it a potential 2D scaffold for a possible adipose and kidney tissue-engineered construct.

A67
PACU VISCERA EXTRACT OBTAINED BY AQUEOUS TWO-PHASE SYSTEM. POTENTIAL APPLICATION IN RECYCLING WASTE

Gómez GN, Acevedo Gómez AV, Nerli BB, Leiva LC.
Lab InPro-FaCENA, IQUIBA-NEA (CONICET-UNNE) e IPROBYQ-CONICET, UNR. E-mail: lauraleiva2004@yahoo.com.ar

Pacú (*Piaractus mesopotamicus*) production represents 40% of northeast fish yield. It is of interest to valorize the current disposal of fish processing through the use of viscera waste, source of enzymes such as trypsin. Aqueous two-phase systems (ATPS) have been successfully used for separation and purification of macromolecules because exhibit multiple advantages: good resolution, high yield, low cost and proteins retain their biological activity. The goal of this work was to analyze the partition of pacú pyloric caeca extract in aqueous two-phase PEG-citrate systems. Preparation of crude alkaline extract was made by mechanical and sonic digestion of pyloric caeca. A two-phase system formed by polyethyleneglycol (PEG) 3350 - citrate, pH 8.4, was used and the partition of alkaline proteins of the extract was assayed. Trypsin activity was determined with the substrate a-Nbenzoyl-DL-arginine-p-nitroanilide (BAPNA) and protein content was estimated by the Warburg and Christian method. Partition coefficients (Kp) were calculated by the ratio of enzyme trypsin activity or total protein between phases. The experiments revealed that partition coefficient of trypsin was greater than unity (Kp_{Trip}: 2,2), while partition coefficient of total proteinswas smaller than one (Kp_{TP}: 0,33). This result indicates a tendency for trypsin-like enzymes to concentrate in the top phase (PEG-enriched phase), while proteins were partitioned to the salt-enriched phase (bottom phase). Thus, a high separating capability was verified in the assayed system. On the other hand, X ray plates were treated with the top phase achieving a complete removed of thegelatine layer which covers it, with no interference of PEG. These studies demonstrate the applicability of a simple procedure to obtaina phase enriched with enzymes from pacú viscera extracts and their use in recycling of radiographic plates.

A68
HYALURONIC ACID SEMI-INTERPENETRATED HYDROGELS FOR ADHESION AND SELECTION OF BULL ESPERMATOZOA

Liaudat AC(1), Blois D(1), Morilla G(1), Capella V(1,2), Rivero R(2), Rivarola C(2), Barbero C(2), Rodríguez N(1), Bosch P(1)
(1)Dpto de Biol Mol y (2) Dpto de Qca, Fac de Cs. Exactas Fco-Qcas y Naturales, UNRC.

Hyaluronic acid (HA) is an important glycosaminoglycan present in oocyte-cumulus cell complex. It is known that sperm with good motility, plasma membrane integrity, nuclear maturity and low DNA damage preferentially bind to HA molecules. The aim of this work was to develop HA-semi-interpenetrated polymeric biomaterials and study the binding ability of bull sperm to these surfaces. Hydrogels were synthesized with HA and the interaction and percentage of sperm attached and released from the surface were investigated. Motility, viability, acrosome membrane integrity and plasma membrane functionality of released sperm were determined. The results indicate that 60% of spermatozoa exposed to HA semi-interpenetrated hydrogels bound to hydrogel surfaces and 70% of attached spermwas released after addition of hyaluronidase. Released sperm had acceptable rectilinear motility, high viability and low acrosome reaction. The plasma membrane integrity of spermatozoa in the released population was similar to that determined in the initial population. In conclusion, these results indicate that HA semi-interpenetrated hydrogels can be used to select high-quality sperm for use in assisted reproduction techniques.

A69
CHARACTERIZATION OF PARTIALLY REPROGRAMMED EQUINE AND BOVINE iPS CELLS IN DIFFERENT CULTURE CONDITIONS

Navarro M, Campetella C, Mutto A, Blüguermann C.
Laboratorio de Biotecnologías Reproductivas, Instituto de Investigaciones Biotecnológicas 'Dr Rodolfo Ugalde' (IIBIO).
E-mail: minavarro@iibintech.com.ar

Induced pluripotent stem (iPS) cells can be produced by introducing four transcriptional factors - Oct4, Klf4, Sox2 and c-Myc - into adult somatic cells. These cells are similar to embryonic stem cells (ESC) considering their gene and protein expression profile. Although reprogramming protocols are well established for murine and human cells, this is not the case for domestic species such as equine and bovine. Taking this into consideration, the aim of this work was to reprogram equine and bovine fibroblast and analyze their pluripotent state in different culture media. We used lentiviral particles carrying STEMCCA plasmid to induce reprogramming. Two days after infection, equine and bovine infected fibroblasts were cultured in different conditions using irradiated murine embryonic fibroblast (iMEF) as a feeder layer. The different culture conditions tested were: DMEM + 10% fetal bovine serum (FBS) (M1), DMEM + 10% FBS + leukemia inhibition factor (LIF) (M2), *knock out* DMEM + *knock out* serum replacement + LIF (M3). To evaluate cells pluripotency, we checked colony morphology, performed a colorimetric alkaline phosphatase assay, and analyzed the expression of endogenous and exogenous pluripotency genes (PG) by PCR. Equine and bovine cells from all conditions started growing as compact colonies with define borders 10 to 15 days after viral infection. In both cases, cells showed a high nuclear/cytoplasmic ratio characteristic of pluripotent cells. However, cells cultured in M1 and M2 lost their morphology after the first passage. On the contrary, clones cultured in M3 were passaged more than 10 times although we could not expand them, with the exception of one equine clone (A5) from which we were able to obtain a large number of cells. Alkaline phosphatase expression was stronger for A5 clone in comparison with other M3 clones tested. When the expression of PG was analyzed, we observed that colonies cultured in M3 expressed all the endogenous PG tested and had silenced the exogenous expression of Oct4 and Sox2 (evaluated at passage 2 and 9) excluding equine clone A5 which retained expression of exogenous Sox2. This clone was also able to differentiate *in vitro* and to express genes from mesoderm and endoderm germ layers. These results indicate that we successfully obtained 'partially' reprogrammed equine and bovine iPS cells in M3 culture conditions. Cells were able to sustain their undifferentiated state only if exogenous PG expression persisted, which is in accordance with previous reports. Once expression from exogenous PG is silenced, endogenous expression of PG is not enough to maintain the pluripotent state of iPS cells cultured in M3 conditions. Based on this, we consider that the main roadblock to obtain 'fully' reprogrammed equine or bovine iPS cells could be surpassed if the appropriate culture conditions are found.

BIOCELL 43 (suppl. 3), 2019 ABSTRACTS
ISSN 0327- 9545 A1 – A104
ISSN 1667-5746 (online version)

A70

DIFFERENTIAL ACTIVITY AND EVALUATION OF PROLIFERATION IN THE OVARY OF A SOUTH AMERICAN BAT, *Eumops patagonicus* (CHIROPTERA: MOLOSSIDAE): PRELIMINARY RESULTS

Rodríguez FE [1,2], Olea GB[2,3], Aguirre MV[3], Lombardo DM[4].
[1] Universidad Nacional del Nordeste. Facultad de Ciencias Exactas y Naturales y Agrimensura. Avenida Libertad 5470, Corrientes. Argentina. [2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). [3]Universidad Nacional del Nordeste. Facultad de Medicina. Laboratorio de Investigaciones Bioquímicas (LIBIM). [4] Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal (INITRA).Chorroarin 280, Buenos Aires, Argentina.
E-mail: florencia0066@yahoo.com.ar

The order Chiroptera comprises a great diversity about reproductive patterns; even we could affirm they enclose all the variations in the mammalians. In the Molossidae order; studies have been done on the structure of the female reproductive system and ovarydynamic in species such as *Molossus fortis*, *Molossus rufus*, *Molossus molossus*, and *Mops condylurus*. A full dominance of right ovary in this order is well known, but the explanation about that it has not yet been known. The present study aimed to characterize the ovarian activity of *Eumops patagonicus* in different seasons and reviewed the proliferative activity in both ovaries.This study was realized using 20 specimens obtaining of wildlife, they were anesthetized and euthanized to isolate the reproductive tract. The material was processed following the conventional technique to obtain histological sections and colored whit hematoxylin-eosin. A part of sections was cut in 3µm and was processed following the immunohistochemistry technique using the streptavidin-biotin method to evaluate the proliferative activity in both ovaries with PCNA antibody (1:100). The right ovary (RO) and left ovary (LO) were analyzed, in both the cortical zone with follicles and the medullary region with scarce interstitial glandular tissue (IGT) was distinguished. Regarding the composition of the cortex, along the seasons, only bare oocytes l forming nests, primordia, primary and secondary follicles were found on LO but no one Graafian follicle. The medullary region was scarce. Nevertheless, on RO it was possible to observe the structures of folliculogenesis as well as a biovular follicle in winter. Concerning the IGT, it was found in greater quantity than the LO but in a lesser proportion to what is described for *M. rufus*. In winter recent pregnant females were found, the RO with a corpus luteum occupying a large part of the ovary. These preliminary results agree with the ovarian and functional asymmetry. In the RO, the stages of folliculogenesis were observed, including a corpus luteum on the same side of the pregnant horn. About the proliferative condition, in both ovaries we could see positive nucleus to PCNA.But in RO was higher in number if compared the same type of follicle. We conclude that *E. patagonicus* presents morphological and functional ovarian asymmetry as well as other members of the order.

A71

USE OF VISCERA EXTRACT FROM SURUBIM (*Pseudoplatystoma corruscans*) FOR THE PRODUCTION OF CASEIN HYDROLYSATE

Van de Velde A, López JM, Sánchez S, Leiva L.
LabInPro. IQUIBA-NEA UNNE-CONICET.E-mail: andrevdev@hotmail.com

Protein hydrolysates are mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources using partial hydrolysis. The method of preference is enzymatic hydrolysis since is easily controllable, quick, specific and it is an affordable technology to produce high value-added products. This method is widely applied, not only to upgrade the functional and nutritional properties of proteins in the food industry, but also is used in other areas of biotechnology such as by providing specialized media for microorganisms grown in the laboratory. Today, the preparation of hydrolysates derived from milk proteins and casein has received much attention due to the diversity and unique functional properties. Proteases used to obtain a more selective hydrolysis of milk proteins are from different sources, between them fish viscera generated during the commercial processing. The Northeast of Argentina has native fish species cultivated, and of total aquaculture annual production, above 3300 tons, approximately 74 tons corresponds to surubím (*Pseudoplatystoma corruscans*). This freshwater fish is carnivorous so the viscera, that constitute the majority waste of processing, is a rich source of proteases. The objective of this work was to study the proteolytic activity of surubim viscera extract on casein. The extract was prepared from tissue that coats the stomach area near duodenum. Previous to proteolytic assays, the thermal stability of enzymatic extract by 2h (0, 8, 25, 37, 45, 50, 55, 60, 75 and 100 °C) and proteases inhibitors (soybean trypsin inhibitor -TBSI-, phenylmethylsulfonyl fluoride -PMSF- and disodium ethylenediaminetetraacetate -EDTA-Na_2-) were assayed over Nα-Benzoyl-dl-arginine-p-nitroanilide (BApNA) as substrate. The proteolytic capacity of the extract was evaluated at 0, 1, 5, 15, 30 and 60 min, on casein. The cleavage of casein was analyzed by SDS-PAGE (14%, Coomassie Blue stain). The thermal stability profile of the viscera extract revealed that these fish enzymes were highly stables at temperatures below 55°C and they retained the 50% of their initial activity when they were incubated at 60 °C. In addition, the activity on BApNA was strongly inhibited by TBSI, whereas PMSF and EDTA-Na_2 did not exhibit an effect on activity. The 60% of proteolytic activity on casein developed in one hour was achieved during the first 5 min. Simultaneously, the extract showed similar behavior by SDS-PAGE analysis. The typical bands of casein (αs1, β and κ) showed rapid degradation in a short incubationtime. The results suggest that trypsin-like enzymes present on surubim viscera extract have high thermal stability. The studies on milk protein demonstrated the ability of the fish viscera extract to producea casein hydrolysate. In this way, the findings presented in the current work demonstrate that the surubim viscera extract could be considered as a potentially strong candidate for future industrial applications, such as the obtaining of milk peptones for the cultivation of microorganisms.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

REPRODUCTION II

A72
BLOCKING GABAB RECEPTORS (GABABR) FROM BIRTH TO WEANING IN MICE INDUCES PROFOUND CHANGES IN THE GONADOTROPIC AXIS

Bizzozzero Hiriart M, Di Giorgio NP, Libertun C and Lux-Lantos V.
Instituto de Biología y Medicina Experimental (IBYME-CONICET, Buenos Aires, Argentina).E-mail: m.bizzozzero@hotmail.com

We have previously shown that the administration of aGABAB antagonist (CGP55845) to neonatal BALB/C mice from postnatal day (PND) 2-6 significantly decreased arcuate nucleus (ARC) kisspeptin (*Kiss1)* expression in both sexes. Furthermore, CGP55845 decreased PND7 BW, increased PND7 and PND21 anogenital index (AGI) and delayed puberty onset in females. Here our aim was to induce a more sustained inhibition of GABABR signaling and evaluate its effects on the gonadotropic axis. Neonatal Balb/c male (M) and female (F) mice were injected with CGP55845 (1 mg/kg, sc, CGP) or saline (Sal) from PND2-21, three times/day (8AM, 1PM, 6 PM) and sacrificed at 3 PM (after two injections on the last day). Serum samples and gonads were collected for hormonal measurements by RIA. Brains were frozen and 500 µm slices were obtained on a cryostat. ARC and antero ventral periventricular nucleus (AVPV) micropunches were obtained. *Kiss1*, kisspeptin receptor *(Kiss1r)*, prodynorfin (*Pdyn)*, neurokinin B (*Tac2)*, tyrosine hydrolxylase *(TH)*, progesterone receptor (*Pgr)*, GABAB1and GABAB2 subunits of the GABABR *(GABAB1, GABAB2)* mRNA expression was assessed in the micropunches by qPCR (control gene: Cyclophilin B). Body weight (BW) and AGI (anogenital distance/BW) were evaluated on PND 7, 14 and 21. In the ARC CGP induced a decrease in *Kiss1* (FCTRL:1,16±0.24 vs FCGP:0,67±0.16 vs MCTRL:1,22±0.24 vs MCGP:0,70±0.13, CGP vs CTRL p<0,04) and an increase in *Pdyn* (FCTRL:0,97±0.26 vs FCGP:1,77±0,49 vs MCTRL: 1,37±0.32 vs MCGP:3,01±0.85, CGP vs CTRL: p<0,02) and *GABAB1 (*FCTRL:0,42±0.08 vs FCGP:1,37±0.43 vs MCTRL:0,74±0.13 vs MCGP:1,38±0.46, CGP vs CTRL:p<0,04*)* in both sexes, while adecreasein *Tac2* was only observed in males (MCTRL:1,01±0.14 vs MCGP:0,45±0.045:p<0,02); no changes were observed in *Kiss1r* or *Pgr* expression. In AVPV no CGP-induced changes in *Kiss1* or *TH* expression were detected. FSH was significantly decreased in CGP-treated males (FSH (ng/ml): MCTRL: 9,3±1,28 vs MCGP:5,78±0.9:p<0,003). Preliminary data suggest that gonadal testosterone content did not differ between treatment groups. BW was significantly decreased by CGP on PND21 in females while this parameter was decreased at all ages studied in males. CGP significantly decreased AGI in both sexes. These results demonstrate that sustained inhibition of GABABR signaling during the critical postnatal period of development and maturation of the gonadotropic axis profoundly alters key ARC gene expression such as *Kiss1*, *Pdyn*, *Tac2*, altering sexual differentiation (AGI) and BW. In addition, in males FSH levels are decreased to female levels in agreement with feminization of AGI. *Supported by CONICET, ANPCYT, UBA, Fundación René Barón, Fundación Williams*.

A73
EFFECTOF TROLOX ON PLASMA MEMBRANE INTEGRITY AND MITOCHONDRIAL FUNCTION IN REFRIGERATED PORCINE SEMEN

García N, Morado S, Cetica P, Córdoba M
Instituto de Investigación y Tecnología en Reproducción Animal (INITRA, UBA),Unidad Ejecutora de Investigaciones en Producción Animal (INPA, UBA-CONICET),Cátedra de Química Biológica, Facultad de Ciencias Veterinarias,Universidad de Buenos Aires, Argentina.E-mail: mcordoba@fvet.uba.ar

Gamete refrigeration is a commonly used biotechnologyforporcine production. Porcine sperm have a high proportion of unsaturated lipids in their plasma membrane making them more susceptible to damages caused by temperature descent. Trolox (6-hydroxi-2,5,7,8-tetrametilchroman-2-ácido carboxilico) is a vitamin E analogue used as antioxidant, which proved to improve sperm quality post-freezing in several species. In the porcine, sperm have a high oxidative metabolism and energy is generated mainly in the mitochondria. The aim of our work was to study the changes in functional parameters in porcine sperm refrigerated with or without Trolox. Sperm capacitation was analyzed using the epifluorescentchlorotetracycline technique (CTC), plasma membrane functionality by hypoosmotic test (HOS-test), sperm vitality using Trypan Blue, acrosome integrity by a differential interferential microscope (DIC), mitochondrial membrane potential by 5,5', 6,6'- tetracloro-1,1', 3,3'yoduro de tetra-etil-benzimidazolil-carbocianina (JC-1) stain and *in vitro* fertilization by the observation of pronuclei formation. Samples were suspended in a commercial diluent in a 1:10 proportion and treated with or without Trolox at 17 ºC. Data were analyzed using ANOVA and Tukey test (P<0.05). In fresh semen, progressive motility was 71.43±8.52%, vigor 3.43± 0.53 and no significant differences were found in membrane integrity compared with refrigerated semen treated with Trolox (P>0.05). Sperm vitality in refrigerated samples was improved by the addition of Trolox (P<0.05) but the capacitation and acrosome reaction percentages remained low as in fresh semen (P>0.05). Regarding mitochondrial membrane potential, the lowest level was observed in samples refrigerated without the antioxidant (P<0.05). In conclusion, Trolox would have a protective effect both by conserving plasma membrane functionality and by maintaining a normal mitochondrial function, thus generating a reduced capacitation and a high vitality percentage in refrigerated porcine semen.

A74
COCULTURE OF PORCINE EMBRYOS AND OVIDUCTAL EPITHELIAL CELLS: A MODEL TO IMPROVE EMBRYO PRODUCTION

Lorenzo MS[1,2], Teplitz GM[1,2], Cruzans PR[1,2], Luchetti CG[1,2], Maruri A[2], Tello, M [2]Lombardo DM[2].
[1]CONICET [2]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal, Cátedra de Histología y Embriología.E-mail: soledadlorenzo@fvet.uba.ar

The coculture of gametes and embryos with somatic cells has been an attempt to improve embryo production. The use of oviductal epithelial cell could mimic the *in vivo* conditions. This study aimed to evaluate if the coculture of porcine embryos and oviductal epithelial cells increase embryo production. Oviducts from slaughteredsows with corpus luteum on their ovaries were dissected, and porcine epithelial oviductal cells (POEC) were obtained by pressing the isthmus surface with a slide. Individual cells were separated by cycles of aspiration-ejection with 21G-needle in a1 mL-syringe and vortex. After 3cycles of decanting in a 15-mL centrifuge tube, they were seeded using DMEM F12 with 10% SFB. Culture medium was changed every 48 h until confluence. POEC were trypsinized and cryopreserved until use. For the passage 1 (P1) culture, 2 days before *in vitro* fertilization (IVF) the POEC were warmed and seeded at a concentration of 50000 cells/mL in 50 uL-drops of NCSU 23 supplemented with 0,5 mM sodium pyruvate, 5 mM sodium lactate and 2,5% SFB. They were cultured in 7% O_2, 5% CO_2 humidified air at 39°C. Cumulus-oocyte complexes were obtainedby follicular aspiration of ovaries from slaughtered sows. They were matured *in vitro* in groups of 50 per well, in 5% CO_2 humidified air at 39°, in modified Medium 199 containing 1mMAMPc and 1,5 UI hMG for the first 22 h and without them for the last 22 h. For IVF, 17°C-refrigerated sperm of proven fertility was centrifuge at 490 x g for 5 minutes and resuspended in Medium 199 supplemented with 0,4% BSA, 5 mM caffeine, 2,9 mM sodium lactate and 1,25 mM sodium pyruvate (IVF media). Denuded oocytes and $1x10^6$ sperm/mL were coincubated in 100 uL-drops of IVF media, for 4 h in 7% O_2, 5% CO_2 humidified air, at 39°C. Presumptive zygotes were washed three times and randomly placed in drops of 50 uL NCSU 23 (with sodium pyruvate and lactate) alone (control, n=97), with POEC-P1 (n= 99) or with POEC-P1 and 2,5% SFB (n= 107). After two days of culture, they were washed and changed to drops containing fresh NCSU 23 (with 5mM glucose) and without POEC-P1. They were cultured up to day 7 in the conditions previously described. Cleavage rate was registered at day 2 and blastocysts rate at day 7. The rate of cleavage was 35,05% (control) 36,36% (POEC-P1) and 38,32% (POEC-P1 2,5% SFB) and it did not differ between categories. The rate of blastocyst was 3% (POEC-P1) and 2% (POEC-P1 2,5% SFB) and it did not differ between the coculture groups ($p>0,05$). We did not obtain blastocyst in control. The coculture of porcine embryos and POEC-P1 during the first 2 days of culture appears to be a model which could improve pig embryo production. The addition of 2,5% SFB during coculture would not affect embryo production, although embryo quality needs to be evaluated.

A75
INVOLVEMENT OF THE NOVEL CANNABINOID RECEPTOR GPR55 IN SPERM FUNCTION IN BOVINES

Lottero RM, Vernaz Z, Alonso CAI, PerezMartinez S.
Lab. de Biología de la Reproducción de Mamíferos (CEFYBO, FMed, UBA-CONICET). E-mail:mv.rlottero@gmail.com

Mammal oviduct acts as a functional reservoir of spermatozoa, providing a proper environment for their maintenance and for competence to oocyte fertilization. Several molecules from the oviductal fluid are involved in sperm- oviduct interaction. Previous results from our laboratory have shown the involvement of endocannabinoid system in sperm capacitation and in sperm-release from oviductal epithelial cells (OECs) in bovines. We previously reported that anandamide (AEA), a known endocannabinoid, fluctuated in the oviductal fluid during the estral cycle in bovines. In addition, AEA induces sperm capacitation and sperm release from OECs through CB1 and TRPV1 cannabinoid receptors activation. In these molecular pathways are involved a calcium increase and cAMP/PKA activation. On the other hand, G-protein-coupled receptor 55 (GPR55) was proposed as a novel component of the endocannabinoid system, which molecular pathway includes a Protein Kinase C (PKC) activation. The aims of this work were to characterize GPR55 receptor in bovine spermatozoa and to study its participation in sperm function. Our studies were performed with bovine cryopreserved spermatozoa (without incubation (T=0), capacitated, and released from OECs) and co-cultures of sperm-OEC. First, we analyzed the presence (by Western Blot) and localization (by immunocytochemistry) of GPR55 in bovine spermatozoa. GPR55 was presented as a band of an apparent molecular of 37 kDa, as expected for this protein. At T=0, it was localized in the acrosomal region of bovine spermatozoa. However, when sperm were released from the oviductal epithelium, the receptor was mainly found in the equatorial segment and flagellum; a similar localization was observed in capacitated sperm. Subsequently, we evaluated the involvement of GPR55 in sperm capacitation induced by AEA by analyzing of increase of tyrosine phosphorylation (pY) levels and substrates of PKA (pPKA), essential events associated with this process. Results indicated that the presence of CID16020046 (GPR55 antagonist) produced a decrease of 15% in pY and ~25% of pPKA levels, suggesting a participation of GPPR55 in sperm capacitation. In addition, we evaluated the involvement of PKC in GPR55 activation during sperm capacitation induced by AEA. The presence of a PKC inhibitor diminished (30%) serine/threonine phosphorylated proteins levels. Considering that released and capacitated spermatozoa presented GPR55 mainly in the flagellum, we studied its participation in motility. Spermatozoa were incubated in capacitating conditions with different concentrations of GPR55 agonist AM251 (10^{-5}-10^{-9} M). Results showed that AM251 (10^{-7} M) induced a significant increase in sperm motility (vigor), similar than that observed in the presence of AEA, which was attenuated with GPR55 antagonist. Overall, results indicate that sperm bovine present a functional GPR55 that may play a role in sperm capacitation and motility, suggesting that its activation would contribute to the acquisition of sperm fertilizing ability in bovine.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A76
CHARACTERIZATION OF VITRIFIED *IN VITRO* PRODUCED BOVINE EMBRYOS

Madrid Gaviria S[1,2], López Herrera A[1,2], Urrego Álvarez R A[3], Restrepo Betancur G[1,4], Echeverri Zuluaga J J[1,2], Niemann H[5]
.[1]Departamento de Producción Animal, Universidad Nacional de Colombia Sede Medellín, [2]Grupo de investigación BIOGEM, [3]Facultad de Ciencias Veterinarias y Zootecnia, Universidad CES, Grupo de investigación INCA-CES, [4]Grupo de investigación GIBA, [5]Institute of Farm Animal Genetics, FLI, Mariensee, Germany.E-mail:smadridg@unal.edu.co

Cryopreservation techniques allow long-term preservation of viable cells, generating a significant impact on assisted reproductive technologies. However, even when these techniques are widely used for embryo cryopreservation, there are still some concerns about the detrimental effects on the physiologic, genetic and epigenetic status of vitrified early embryos. The aim of this study was to evaluate if vitrification and warming process may affect cell number and distribution, and gene expression profile in *in vitro* produced bovine embryos. Abattoir-derived oocytes were matured, fertilized and cultured *in vitro* according to a standard procedure. On day 7, blastocysts were vitrified using the minimum volume vitrification method. After warming, cell number and distribution were analyzed by differential staining of the inner cell mass (ICM) and the trophectoderm (TE), while the transcription level of developmental important genes was analyzed using quantitative reverse transcription PCR (RT-qPCR). Day 7 expanded blastocyst presented a survival rate of 90.4% after vitrification and warming process. There were no significant differences for total cell number (TCN) and their distribution in the ICM and TE between fresh and vitrified embryos ($P<0.05$) (TCN: 146.5 ± 8.5, 150.9 ± 6.05, ICM: 52.6 ± 3.3, 51.7 ± 4.5, TE: 146.5 ± 8.5, 150.9 ± 6.0 for fresh and vitrified embryos, respectively). Regarding to the gene expression profile, no differences were found for the studied genes between fresh and vitrified embryos, however, in those embryos that did not survived the cryopreservation process there was a significant decreased in the expression of OCT4. These results show that vitrification and warming using the minimum volume method is a reliable option for long-term storage of *in vitro* produced bovine embryos, since this method allows the maintaining of the initialembryo quality after the cryopreservation process.

A77
LOW LEVEL LASER THERAPY (LLLT) REDUCES CHEMOTHERAPY-INDUCED OVARIAN DAMAGE

Oubiña G[1], Pascuali N[1], Scotti L[1], Negri Montes L[1], Velázquez C[1], Higuera J[2], Abramovich D[1], Parborell F[1].
[1]Laboratorio de Estudios de la Fisiopatología del Ovario (IBYME-CONICET), Buenos Aires, Argentina. [2]Higuera Dental Practice, Buenos Aires, Argentina.E-mail: fparborell@gmail.com

It is known that LLLT has beneficial effects on several processes including wound healing, pain and inflammation. LLLT modulates biological functions, including cell proliferation, apoptosis and angiogenesis, which in the ovary are intimately related to fertility. Premature Ovarian Failure (POF) is characterized by the disappearance of ovarian follicles in young women, which may be caused by chemotherapy. Current treatments for POF, mainly hormone therapies, are not completely effective. The present study proposes photobiomodulation as a strategy to protect the ovary in patients undergoing chemotherapy. The objective was to analyze the effect of LLLT in a) a chemotherapy-induced POF model in adult mice and in b) rat granulosa cell culture exposed to chemotherapy. For a), POF was induced with cyclophosphamide (CTX, 75mg/weight) in F1 mice (8 weeks) and LLLT (8, 12 and 16 Joule) was applied. For b), a rat primary granulosa cell culture in the presence of doxorrubicin (DX, 10 uM) was performed and LLLT (8, 12 and 16 J) was applied. To characterize ovarian morphology of our experimental groups, a quantification of follicular structures was performed. CTX increased the % of atretic follicles and diminished the % of primary and preantral follicles compared to control group ($p<0.05$). However, LLLT(8 J) increased the %of antral follicles and decreased the % of atretic follicles compared to CTX group ($p<0.05$). No differences were observed in higher doses of LLLT (12 and 16J). These results were corroborated by IHC for Anti-Müllerian Hormone (AMH). To evaluate the ovarian reserve, a quantification of primordial follicles in ovarian sections stained with anti-DDX4 was performed. CTX reduced the number of primordial follicles ($p<0.05$), while the LLLT (8J) restored this value to control. To evaluate the effect of LLLT (8J) on ovarian apoptosis, a TUNEL assay was performed on ovarian sections. CTX increased the % of TUNEL-positive mature antral follicles compared to control group ($p<0.05$), while LLLT (8J) restored it to control. Additionally, CTX decreased the proliferation index of granulosa cells compared to control group ($p<0.05$) but LLLT did not restore it. To analyze the in vitro effect of LLLT in the presence of DX (doxorubicin) on viability and apoptosis in rat granulosa cells, MTS and Annexin assays were performed respectively. DX reduced viability compared to control ($p<0.05$) but LLLT (4, 8 and 16 J) did not restore it. Additionally, apoptosis was evaluated by flow citometry. DX increased apoptosis compared to control ($p<0.05$), while LLLT (16 J) reduced apoptosis compared to DX group ($p<0.05$). These results show that LLLT "photobiomodulates" folliculogenesis and atresia in the POF model. Additionally, LLLT was able to decrease apoptosis induced by DX in granulosa cell. In conclusion, LLLT might become a novel and useful tool in the treatment of POF induced by chemotherapy.

BIOCELL 43 (suppl. 3), 2019 ABSTRACTS
ISSN 0327- 9545 A1 – A104
ISSN 1667-5746 (online version)

ECOTOXICOLOGY AND TOXICOLOGY

A78
LETHAL AND SUBLETHAL EFFECTS OF THE COMMERCIAL FORMULATION ZERO® (5% LAMBDA-CYHALOTHRIN) ON TADPOLES OF *Ceratophrys ornata*

Barreto E, Pantucci, MJ; Rimoldi F, Salgado Costa C, Venturino A, Natale GS.
CIM,Fac. Cs. Exactas, UNLP, La Plata, Argentina; CONICET y CITAAC, Univ. Nac. del Comahue.
E-mail: gnatale@quimica.unlp.edu.ar

Lambda-cyhalothrin (LCT) is a synthetic pyrethroid insecticide commonly used in the pampean region (central eastern Argentina) to control agricultural pests, and it is classified as very toxic to aquatic organisms. The effects of this insecticide on non-target biota have been reported meanly in invertebrates and fishes. This study assessed lethal and sublethal effects of the commercial product Zero® (5% of LCT) on the tadpoles of the native horned frog *Ceratophrys ornata*. Spawning was induced by the Amphiplex method and eggs were maintained in plastic trays until individuals reached Gosner stage 31. The experimental design consisted of exposing organisms individually in 200 ml glass chambers during 96 h. Final nominal LCT concentrations ranged from 0.01 to 2.5 ug/L and all solutions were fully renewed every 24 h. Tests included 10 replicates per concentration and a negative control group (water without insecticide). Lethal and sublethal endpoints were recorded every 24 h, including mortality, swimming activity and the presence of morphologic abnormalities. Also, tadpoles were recorded at 96 h of exposure, while emitting sounds, in an acoustic room and with a calibrated system (directional microphone connected to an audio plate). For each recorded sound, three bioacoustic variables were determined: sound duration (s), number of pulses and dominant frequency (Hz). The LC-50/EC-50, LOEC and NOEC for each endpoint were obtained by PROBIT analysis. One-way ANOVA with Dunnett tests were performed to compare treatments with the control group. Results showed significant high sensitivity ($p < 0.05$) of *C. ornata* to this insecticide compared with other anuran species like *Xenopus laevis* and *Boana pulchella* (unpublished data). Bioacoustic effects were detected at lower concentrations than other endpoints. This study provides evidence in favor of using the species as a valid model in ecotoxicology. We suggest that tadpole vocalizations are a sensitive and useful non-lethal endpoint to evaluate the effects of very toxic insecticides.

A79
IN-VITRO EXPOSURE TO BISPHENOL A INCREASES GFAPGENE EXPRESSION IN WHOLE HYPOTHALAMI ANDIL-18 GENE EXPRESSION IN GT1-7 CELLS

Fernandez M[1], Marigliano C[1], Riaño-Gomez J[1], Libertun C[1,2] and Lux-Lantos V[1].
1-Laboratorio de Neuroendocrinología-Instituto de Biología y Medicina Experimental (IByME-CONICET) and 2-Facultad de Medicina-UBA.E-mail: mfernandez@dna.uba.ar

Bisphenol A (BPA), a monomer of polycarbonate plastics, and Benzophenones (BPs), UV-filters, are endocrine disrupting chemicals (EDC) found in everyday products. In this study we analyzed the effects of the exposure to BPA, BP2 and BP3 on Glial fibrillary acidic protein (GFAP) and IL-18 (a pro-inflammatory cytokine expressed in GnRH neurons) in whole hypothalami isolated from adult Balb/c males and IL-18 in mature GnRH neurons (GT1-7 cells, donated by Dr. Pamela Mellon, UCSD). Hypothalami were incubated in Krebs-Ringer medium in the presence or absence of BPA, BP2 or BP3 ($1x10^{-9}$M) or medium alone (C) for six hours. After the incubation, hypothalami were homogenized in Tri-Reagent (Molecular Research Center, OH, USA) and mRNA extracted for *Gfap*and*Il-18* analysis by qPCR. GT1-7 cells were cultured in 12-well plates (200000 cells/well) in DMEM supplemented with 10% FBS, pyruvate and Penicillin/Streptomicin (complete medium). After 24 hours, media were changed to DMEM without phenol red, supplemented with 10% charcoal-stripped FBS, pyruvate and Penicillin/Streptomicin (stimulation medium). Cells were incubated with BPA, BP2, BP3 ($1x10^{-9}$M) or vehicle (C) in stimulation media for 24 hours. RNA was extracted using Tri-Reagent and *Il-18* analyzed by qPCR. RNA (1□g) was reverse transcribed and qPCR performed using specific primers. Hypothalami exposed to BPA showed increased *Gfap*gene expression (C=0.82±0.16, BPA=1.70±0.39; ANOVA $p<0.05$, n=9), whereas neither BP2 nor BP3 had any effect (Anova: ns). There was no change in *Il-18* gene expression in whole hypothalamiwith the exposure to any of the EDC. Twenty-four-hourexposure to BPA increased *Il-18* gene expression in GT1-7 cells (C=1.03±0.04, BPA=1.25±0.11; Repeated measures Anova, $p<0.05$, n=7), whereas exposure to BP2 and BP3 did not result in *Il-18* significant increase (C=1.03±0.04, BP2=1.25±0.22, BP3=1.34±0.20; Repeated measures Anova: ns, n=7). Our results suggest that exposure to BPA could directly increase astrocyte activation and gene expression ofinflammatory molecules in GnRH neurons. Further studies are needed to dissect the mechanisms involved. Supported by: CONICET, ANPCyT, UBA, International Society for Neurochemistry, Asociación ORT Argentina, Fundación René Barón, Fundación Williams.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A80
UNDERSTANDING THE MECHANISM OF SILVER NANOPARTICLE TOXICITY

Garcés M, Magnani N, Calabró V, Marchini T, Cáceres L, Salgueiro J, Galdoporpora J, Zubillaga M, Moretton M, Desimone M, Alvarez S, Valacchi G, Evelson P.
Universidad de Buenos Aires, IBIMOL UBA-CONICET, Cátedra de Química General e Inorgánica, Facultad deFarmacia y Bioquímica. E-mail: msgarces87@gmail.com

Along with the development of silver nanoparticles (AgNP) applications, the concern about their possible toxicity has increasingly gained attention. As the respiratory system in one of the main route of exposure, the aim of this study was to evaluate the harmful effects developed in lung after an acute AgNP exposure using 2D and 3D *in vitro* and *in vivo* models.When AgNP were characterized they showed a hydrodynamic diameter of 17±6 nm and they were able to initiate the hemolytic cleavage of H_2O_2 that may lead to $OH^{\bullet}$ production. First, an *in vitro* approach was done in A549 cells exposed to 2.5 µg/mL AgNP up to 24 h. A decreased in mitochondrial respiration ($p<0.01$) and in the extracellular acidification rate under stressed conditions ($p<0.05$) were observed after 3h of exposure, while a 72% increase in H_2O_2 production was observed after 1h ($p<0.001$). Moreover, increased expression of HO-1 was observed after 24 h exposure (49% $p<0.01$). In an EpiAir way 3D tissue exposed to 2.5 µg/ml AgNP for 24 h we observed a decreased in the transepithelial electrical resistance ($p<0.05$).In the *in vivo* studies, Balb/c mice (25g) were intranasally instilled (0.1 mg AgNP/kg body weight). Biodistribution was evaluated by labelling AgNP with ^{99m}Tc showing the lung as the main organ of AgNP deposition. Samples were collected 1 h after the exposure to measure lung O_2 metabolism. Tissue O_2 consumption increased by 31% ($p<0.05$), due to an increased mitochondrial active respiration (55%, $p<0.001$). Moreover, mitochondrial H_2O_2 production rate was also increased by 39% ($p<0.05$) along withan increased SOD and CAT activity (68%, $p<0.01$; 18% $p<0.01$ respectively) and a decreased GSH/GSSG ratio. Taken together, these results show that AgNP remain in the lung, may lead to damage and impaired lung function due to O_2 metabolism alterations.

A81
COMPARATIVE STUDY OF PROTEOLYTIC ACTIVITIES OF VENOM FROM ADULT AND JUVENILE SPECIES OF *Bothrops alternatus*

Garcia Denegri ME[1,2]; Van de Velde A[1]; Gómez G[1]; Nuñez Otto B[2]; Sandra[2]; Leiva L[1]; Teibler P[2],
[1] LabInPro, [2] FCV, UNNE,E-mail: garciadenegri@gmail.com

Because of the technological advance in proteomic essays, ontogenic variations studies about snake venoms have become more relevant in the last years. The differences would be associated with differences in the snake venom protein composition and could have importance in the selection of the samples for production and control of quality of antivenoms. On the other hand, some authors suggested the use of venom from young snakes (yV) in order to purify some of the main components present in major proportions than in venom from adult snakes (aV). In this study, we compared venoms from *Bothrops alternatus* (yarará grande) from both stages of ontogeny, focusing on the study of the protein bands by SDS-PAGE and also on the study of proteolytic activities (metalloproteinases and serineproteases enzymes) exhibited by these venoms as the main responsible for the physiopathological action exhibited by this animal. Proteolytic activity was assessing by azocasein method, coagulant activity by citrated plasma and then, amidolyticactitivty was evaluated on BApNA. The results showed differences not only in the protein profile, but also in the enzymatic activities from young and adult snake venoms. Young snake venom coagulated the citrated plasma in the half time (6.3 s) the adult snake venom did. The relation yV/aV of proteolytic activity assessed on azocasein was 2 and 23 in the case of amydolitc action. These results highlight the differences in metalloproteinases concentration (responsible of caseinolytic activity) and especially in the serineproteinases composition (tested on BApNA), which would express in low quantities in adult snake venoms. These evidences warn about the necessity to assay the level of protection that the standart antivenom produces against a potential snakebite by young species and, at the same time, look for the development of antivenoms with pools including young and adult organisms.

A82
FLUOXETINE EFFECT ON LIFE CYCLE AND SOME MORPHOLOGICAL PARAMETERS OF *Dermestes maculatus* (COLEOPTERA: DERMESTIDAE)

Zanetti NI[a], Ferrero AA[a], Centeno ND[b].
[a]Laboratorio de Invertebrados II, Instituto de Investigaciones Biológicas y Biomédicas del Sur (CONICET-UNS), San Juan 670, Bahía Blanca (8000), Provincia de Buenos Aires, Argentina. [b]Laboratorio de Entomología Aplicada y Forense, Departamento de Ciencia y Técnica, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal (1876), Provincia de Buenos Aires, Argentina.
E-mail: noeinesz@yahoo.com.ar

The study of cadaveric insects for detecting xenobiotics in a qualitative and quantitative way is called forensic entomotoxicology. It also studies how xenobiotics can affect insect development. Generally, antidepressants are the most chemical agents used in suicide. In a previous study was determinate and quantified fluoxetine (selective inhibitor serotonine recapture, SSRI) in *Dermestes maculatus* (Coleoptera: Dermestidae), an insect of forensic interest. In this study was evaluated the effect of fluoxetine on *D. maculatus* development. For that, adults of this species were put inside plastic containers and fed with a mixture of pig muscle and fluoxetine

(treatment) considering a 2000 mg/kg concentration to emulate lethal overdose for humans and animals. As control were used beetles fed only with pig muscle. The containers were maintained in an incubator at 30°C±1°C, 55.4% relative humidity and 12:12 h Light:Dark photoperiod. Observations and quantifications were performed diary from the beginning until ending life cycle of beetles. Larvae of each instar, pupae and adults were collected to register total body length, and in the case of larvae, were also registered cephalic width and weight. Durations of each larval instar, each stage of development and total cycle were registered. Two replicates were performed. Larval mortality was not affected by treatment, no differences were found between control and treatment. It was not found a notorious and systematic difference in the duration of egg stage that suggested a treatment effect. From larval instar 2 to larval instar 5 (L2-L5) was observed a systematic reduction of larval instar duration due to treatment. The durations of the larval stage and pupal stage were slightly short in treatment than in control. The total duration of the life cycle was almost the same in the treatment and control. When larval weight was analyzed it was not observed a systematic difference between control and treatment but was observed a systematic increase in larval length due to treatment. With respect to cephalic width, it could not be discarded an effect of treatment and if this is the case, the cephalic width would be major particularly in the last instars. The results would suggest that fluoxetine could produce alterations on *D. maculatus* development, in duration and some morphological aspects, although the total duration of the life cycle would not be altered. Because the results are based in two replicates or trials, the results will be verified with a third replicate that is being performed.

ANIMAL AND PLANT BIOLOGY AND BIOTECHNOLOGY III

A83
Senecio grisebachii BAKER (ASTERACEAE) LEAF EXTRACT: EFFECTS ON THE PEROXIDATION OF MICROSOME MEMBRANES FROM RAT LIVER

[1]Becerra V,[2]Barberón J, [2]Leggieri M, [2]Raineri E, [2]Zeinsteger P, [2]Palacios, A
.[1]Cátedra Biología. Facultad de Ciencias Veterinarias – UCCuyo Sede San Luis, San Luis [2]Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. E-mail: jbarberon@fcv.unlp.edu.ar

Senecio grisebachii Baker ("margarita de campo", Asteraceae, SG) is a 20-80 cm height bush with serrated leaves and yellow daisy-like flowers. Pyrrolizidine hepatotoxic alkaloids (PAs) have been identified in the plant and have caused poisoning when eaten by cattle. Despite this, many *Senecio* spp. are used by native people from Latin America as grounded leaves or flowers mixed with creams or as decoctions to heal skin injuries. Considering the latter, a methanolic extract from dried leaves of SG was studied for its phytochemistry and peroxidation activity on microsome membranes of hepatocytes from Wistar AH/HOK rats. A series of tests were performed on the extract to determine flavonoids, phenolic compound, lipids, carbohydrates and alkaloids. PAs were detected by a field test with Erhlich reagent and characterized by thin layer chromatography (TLC). Chemiluminescence and peroxidation were initiated by adding ascorbate to microsomes. Microsomal protein (1 mg) with the addition of SG extract (0.05, 0.10, 0.20, 0.40, 0.80 and 1.6 mg) was incubated at 37 °C for 180 minutes in 0.01 M phosphate buffer pH 7.4 and 0.4 mM ascorbate. Controls with ascorbate but without extract were used. Phytochemistry demonstrated that flavonoids and phenolic compounds were the more concentrated, while TLC demonstrated the presence of one purple spot with similar Rf to retrorsine standard. Regarding peroxidation, counts per minute (cpm) originated from the light emission from microsomes incubated with SG was statistically lower (concentration dependent) when compared to controls, demonstrating protection against peroxidation. Some *Senecio* species are toxic when ingested by both humans and animals due to liver bioactivation of PAs such as retrorsine, which is present in our SG methanolic extract. However, when applied to the skin, the phenolic and flavonoids present in the leaves exert antioxidant properties, this may explain the folk use of some *Senecio* species.

A84
IMPROVEMENT OF SOYBEAN NODULATION BY BACTERIAL EXPOSURE TO NANOPARTICLES

De Valois N [(1)], Zawoznik M S [(1)], Groppa M D [(1,2)], Benavides M P [(1,2)], Iannone M F [(1,2)]*
*[(1)]Cátedra de Química Biológica Vegetal, Facultad de Farmacia y Bioquímica, UBA, CABA, Argentina. [(2)]IQUIFIB (UBA-CONICET), Facultad de Farmacia y Bioquímica, UBA. CABA, Argentina. *Email: mflorenciaiannone@gmail.com*

In the last years, there was a growing interest in the use of nanotechnology products to improve crop production. Since *Rhizobium*-legume symbiosis is a relevant system in agriculture, the goal of this work was to carry out basic studies to analyze the effects of magnetite nanoparticles (NPs) on soybean-*Bradyrhizobium japonicum* symbiotic association. Soybean seeds were inoculated with *B. japonicum* cells (Control: C) or with *B japonicum* cells exposed during multiplication to 10 ppm of magnetite NPs (BANP). *B. japonicum* used for inoculation was grown for 5 days in a rotary shaker with or without NPs. CFU were adjusted before inoculation. Seeds were left in contact with bacterial suspensions (C or BANP) for 12 hours. Soybean plants were grown on soil in a growth chamber, with periodic water irrigation, and harvested 20 or 30 days later. Bacterial NPs pretreatment improved the germination rate. Additionally, root and aerial part length and total biomass were significantly greater in BANP plants. In the same way, root surface

increased four times and an increment of 13% in chlorophyll content was observed in BANP treatment with respect to controls at any time studied. At 20 days, nodules were present only in BANP treatment. At day 30, leghemoglobin content in the nodules of BANP plants doubled that of the controls. We concluded that incubation of bacteria in the presence of magnetite NPs resulted in improvement of nodulation and biological nitrogen fixation, which was accompanied by greater seedling growth and chlorophyll level. Thus, magnetite NPs could become a good candidate for the design of new products for agricultural use.

A85
EFFECTS OF TWO BIOCHAR TYPES ON ENZYMATIC ACTIVITY IN THREE AGRICULTURAL SOILS WITH DIFFERENT DEGREES OF DEGRADATION

Dominchin MF, Verdenelli RA, Berger MG, Aoki A, Meriles JM.
Instituto Multidisciplinario de Biología Vegetal (IMBIV- CONICET) Córdoba, Argentina. Instituto de Ciencia y Tecnología de los Alimentos (ICTA) Facultad de Ciencias Exactas, Físicas y Naturales (FCEFyN) Universidad Nacional de Córdoba, Argentina.
E-mail: flor.dominchin@gmail.com

Land degradation or soil quality deterioration is an environmental problem with a significant influence on production. Several factors may be cited as causes of land degradation; however, changes in land use and crop managements are currently one of the main drivers of soil disturbance. Particularly, in Argentina agriculture intensification and application of inadequate crop management practices have affected soil quality and productive potential of soils. Biochar, a carbon-rich material produced by thermal decomposition of biomass under oxygen-limited conditions, can increase soil productivity by improving both chemical and physical soil properties. In the present study two types of biochar were used. Thus, the conducted research aimed at assessing the effect of poultry litter and peanut shell biochar on the enzymatic activity in agricultural soils with different degrees of degradation. Typical Haplustoll soils were sampled in Córdoba Province (31°19' S; 64°13' W), Argentina. Three test sites were selected: 1) native forest as a control (L1), 2) lot with 2: 1 rotation (soybean-corn) and no-till (L2) and 3) lot with soy monoculture under conventional tillage (L3). The edaphic deterioration gradient was categorized according to the OM content. Soils were treated with three doses of biochar (0%, 1% and 3%) and incubated for 120 days. Enzymatic activity of the microbial communities was evaluated by hydrolysis of fluorescein diacetate (FDA), dehydrogenase activity (DHA) and β-glucosidase activity (estimated by multiple substrates –MUF-). For L2 and L3 soils, amendment with 3% of quail excreta biochar or 1% of peanut peels biochar significantly increased FDA activity. DHA was higher in L2 soil with 0% and 1% of quail excreta biochar. However, the application of peanut shells biochar did not affect DHA. Finally, β-glucosidase activity increased significantly in L1 soil compared with L2 and L3 soils. At dose of 1% and 3%, both quail excreta and peanut shell biochar, increased β-glucosidase activity. Biochar application modifies enzymatic activity in soils with different degrees of deterioration, principally in those with less OM content. A greater influence of biochar application in total microbial activity (FDA) and β-glucosidase was observed. No major differences were observed between different biochar types.

A86
Bothrops alternatus VENOM PRETREATED WITH CHELATING AGENT FOR ANTISERUM PRODUCTION

López G, Van de Velde A, Leiva L, Fusco LS.
Laboratorio de Investigación en Proteínas (LabInPro), Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste (UNNE).E-mail: giselopezyaccuzzi@gmail.com

Snakebite envenoming is a major public health problem in tropical countries. The only specific therapies currently available for the treatment are antivenoms, which consist of polyclonal immunoglobulins purified from sera/plasma of horses or sheep immunized with doses sublethal of snake venom(s). *Bothrops alternatus* (BaV) venom induces a prominent tissue local damage, especially, hemorrhage, muscle damage and inflammation. These complex pathological phenomena are due to the concomitant action of metalloproteinases (SVMPs) in venoms and others edema-inducing components. In order to obtain a high titer of antibodies and reduced local damage in the animal, an alternative immunization protocol was proposed where the SVMPs action was blocked by using a chelating agent. For this proposal, the *B. alternatus* venom (10 mg/mL) was blocked by EDTA-Na_2 (10 mM, BaV/ EDTA-Na_2) and used as antigen. Previously to the inoculation, the excess of chelating was removed by passing the mixture on Sephadex G-25 column (venom without inhibitor was subjected to the same process) and the effective neutralization of SVMPs using azocasein as substrate was determined. Group of 5 Balb/c mice were immunized subcutaneously on days 0, 15 and 30 with BaV (15-30-45μg) or BaV/ EDTA-Na_2 (45-90-135 μg) emulsified with Freund's Adyuvant (complete first and incomplete-booster). Blood samples were collected by the animals tail tip on days 14, 29 and 41 of protocol immunization and it was destined to ELISA´s test. The results showed that the immunized animal with BaV/ EDTA-Na_2had a higher titer ($5.1x10^4$) than those treated to BaV ($1.3x10^4$). Macroscopic analysis at the inoculation site of mice injected with Freund's adjuvant showed local damage (with non-infectious abscesses) and hypertrophy of inguinal lymph nodes. Our results show that BaV/ EDTA-Na_2formulation, where the SVMPs are blocked, produced a higher humoral response compared with the produced by BaV. These preliminary results demonstrated the potential use of blocking the toxins with chelate to produce antivenom with less damage in the animals.

A87
CHARACTERIZATION OF ALKALINE AND ACIDIC PROTEOLYTIC ENZYMES FROM PALOMETA (*Pygocentrus nattereri*) VISCERA

Medina DM, Acevedo Gomez AV, Leiva LC, Gómez GN.
LabInPro-FaCENA, IQUIBA-NEA (CONICET-UNNE).E-mail: bioq_g@yahoo.com.ar

Fish viscera are regarded as a source of enzymes (proteases) with high commercial value and many industrial and scientific applications. Palometa (*Pygocentrus nattereri*) is widely distributed throughout Paraná River and its proteases were not study until the present. The objective of this work was to study the enzymatic activity and stability of trypsin and pepsin enriched extracts obtained from pyloric caeca and gastric mucosa tissues. Samples were provided from local farmers. Preparations of extracts were made by mechanical and sonic digestion of tissues with the addition of appropriates buffers (1:5 tissue/buffer). Trypsin extract (alkaline) proteolytic activity was assayed with using a-N benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate, while pepsin extract (acid) activity was estimated by acid haemoglobin method. Enzymatic activity and stability were evaluated under different conditions of pH (1-12), temperature (0-80ºC). Alkaline extract exhibited the highest activity at 50-60 ºC-pH 11-12, showed pH stability between 2 and 12 and remained thermostable until 60°C. Acid extract showed its optimum activity at pH 1-2, and 45°C, was stable at a pH range of 1-6 and at temperatures below 45°C. This information will be primordial for ulterior purification process of trypsin and pepsin and the obtainment of high quality enzymes. These extracts present attractive enzymatic properties, so they could be used for industrial applications, such as additive for detergent laundry and collagen extraction for alkaline and acid extract, respectively.

A88
THE PEROXIDATION OF RAT LIVER MICROSOMES: HONEY AS ANTIOXIDANT, A PRELIMINARY STUDY

Ventura M, Barberón J, Yamul D, Góngora A, Marmunti M, Gavazza M, Savignone C, Zeinsteger P, Palacios, A.
Cátedra Bioquímica, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. E-mail: belenventura87@gmail.com

Honey is a substance produced by bees and other social insects, from nectar or molasses that they gather on living plants and that transform or elaborate by evaporating water together with the action of enzymes, segregated by them, and then stored in the alveoli or honeycomb cells. Various studies indicate that honey has chemopreventive and immunoregulatoryeffectsand that is a potential and natural antioxidant food. The objective of this study was to investigate the antioxidant effect of honey on the peroxidation of hepatic microsomes membranes. Rat liver microsomes were incubated with different concentrations of honey (25, 50, 100, 200 mg) in an *in vitro* non-enzymatic ascorbic acid-Fe^{+2} system in order to determine the oxidative effect on membranes and to quantify the peroxidation level in standardized conditions. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Microsomal membranes without honey were used as controls. When considering the effect of honey it was observed that the total cpm/mg protein originated from light emission: chemiluminescence was statistically lower in samples obtained from honey group compared to the control group (without honey). This antioxidant effect was found to be concentration-dependent.

NEW TECHNOLOGIES II

A89
COMPARATIVE STUDY OF THE EVOLUTION OF SIGNS AND SYMPTOMS IN FUNCTIONAL DISORDERS OF THE TEMPOROMANDIBULAR JOINT IN PATIENTS TREATED WITH LOW INTENSITY LASER

Alessandria MV, Olobardi BM, Ficarrotta AO, Ishikawa I, Higuera J.
E-mail: victoriaalessandria@yahoo.com.ar

Temporomandibular joint (TMJ), bicondylar, connects mandible with skull base. TMJ is composed of glenoid cavity, with the articular tubercle of the temporal bone and the mandibular condyle. Between both surfaces is the articular disc, whose peculiarity is to be a fibrocartilage. This allows to adapt to function, remodeling itself through the adhesion of collagen fibers with the loads it receives. The signs and symptoms of TMJ disorders may include pain or tenderness of jaw, pain in one or both of temporomandibular joints and/or ears, difficulty chewing or pain while chewing, aching facial pain and locking of the joint. TMJ disorders can also cause a clicking sound or grating sensation when mouth is open. The objective of this study is to evaluate the evolution of the most frequent signs and symptoms in patients with functional TMJ disorders, treated with an orthopedic plate associated to the application of Low Level Laser Therapy (LLLT) of 910 nm. The signs and symptoms evaluated were joint noise, local pain and decreased mouth opening. The results reflected relief in local pain and absence of joint noise in patients who received LLLT as well as an increase in mouth opening in patients treated with conventional treatment and LLLT.

A90
APPROACH TO DELIVER ANTIOXIDANTS WITH SILICA NANOPARTICLES INTO CELLS

Frontera E[1], *Mitarotonda R*[1,2], *De Marzi M*[1,3], *Guerra LN*[1,3,4].
[1]*Departamento de Ciencias Básicas-Universidad Nacional de Luján;* [2]*FFyB-UBA; 3. INEDES-CONICET;4. FCEyN-UBA.*
E-mail: lguerra@unlu.edu.ar

We showed that antioxidant N-acetylcysteine (NAC) inhibits cellular lipid accumulation during adipocyte differentiation, we carried out in vitro assays in which 0,01 mM to 5 mM NAC doses were added to culture media of the preadipocyte cell line 3T3-L1 (Soto et al, 2016). Here we evaluated Oil-Red-O stained lipid content in 5mM NAC treated adipocytes (ACN) compared to adipocytes (AC), which is set to 100 (100±4 [AC] *vs* 80±2 [ACN] arbitrary units (AU), $p< 0.05$). NAC cellular uptake was 17.4% of total NAC added for 5 mM treatment; with lower doses treatments, 5% of total NAC was absorbed by the cells. We developed 5 mM NAC encapsulated silica nanoparticles ($NPsSiO_2$-NAC) and evaluated their cellular toxicity on 3T3-L1, by colorimetric test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. We prepared silica nanoparticles ($NPsSiO_2$) using organic mold with tetraethylorthosilicate as precursor. We obtained spherical porous $NPsSiO_2$, size of 20 ± 4.5 nm, with an inner hollow of 16 ± 1.3 nm. These $NPsSiO_2$ were a homogeneous population (dynamic light scattering analysis), potential Z negatively charged. We prepared $NPsSiO_2$-NAC by 2 procedures, percentage of loaded NAC: (1) 32.7 ± 2.3% (constant agitation at 500 rpm at 4 °C) and, (2) 96.4 ± 3.1% (sonication pulses at room temperature). NAC release was completed within 28 h. We evaluated $NPsSiO_2$-NAC from procedure (2); samples of 0.25 mg/mL of $NPsSiO_2$-NAC showed cytotoxicity of 84% (OD: 0.11±0.01 [$NPsSiO_2$-NAC-0.25] *vs* 0.71±0.04 [nontoxic control], $p < 0.01$). Samples of 0.05 mg/mL showed lower cytotoxicity such as 57.75%, but only with a cover (8 nm) of bovine sera albumin we obtained nontoxic $NPsSiO_2$-NAC (OD: 0.91±0.09 [$NPsSiO_2$-NAC-0.05] *vs* 0.90±0.08 [nontoxic control], no significant difference). This tool for drug delivery could increase cellular NAC absorption and its anti-lipogenic effect.

A91
EVALUATION OF PATIENTS WITH ORAL MUCOSITIS FOLLOWING ONCOLOGICAL TREATMENT AND TREATED WITH LOW INTENSITY LASER

Giacco CA, Castro S, Flück V, Martinez JE, Higuera J.
E-mail: claugiacco@gmail.com

Oral mucositis (OM) is the secondary reaction to chemotherapy (CT) and/or radiotherapy (RT) that is characterized by the presence of erythematous areas and ulcerative lesions in the buccal mucosa, causing pain and limitations in diet, being one of the most important and common side effects in oncological treatment. More than 90% of patients with RT treatment in head and neck can present this complication. The World Health Organization (WHO) classifies OM in: Grade 0 (no subjective or objective evidence of mucositis), Grade 1 (oral pain with or without erythema, without ulcers), Grade 2 (erythema and ulceration, can swallow solids), Grade 3 (erythema and ulceration and can not swallow solids), Grade 4 (erythema and ulceration and can not feed). Low Level Laser therapy (LLLT) is suggested as a coadjuvant in the treatment and prevention of OM with positive results. The Multinational Association for the Treatment of Cancer Support/International Society of Oral Oncology (MASCC / ISOO) recommends the use of LLLT in prevention and treatment of OM in adult patients who received therapy with high doses of chemotherapy, with or without total body radiation To help prevent the partial or complete interruption of oncological treatment, the onset of OM contributes to weight loss, depression, decreased quality of life of the patient while increasing the cost of maintaining health. The objective of the present study is to evaluate the results of the use of LLLT in patients with indication of CT and/or RT in head and neck region due to squamous cell carcinoma. LLLT was used in a double wavelength (660 nm + 808 nm) and 100 mW of power each with an energy of 8 to 12 J per application point for 80 to 120 seconds until the symptomatology of the patient was eliminated. Approximately 16% of patients who received RT in head and neck cancer were hospitalized by MO. In addition, 11% of patients who received RT due to head and neck cancer had unplanned ruptures in RT due to the presence of MO. No patient in the present study treated with LLLT had catheter placement or gastric button and all of the patients maintained semisolid feeding and the oncological treatment was not interrupted in any case.

A92
IONIC DISSOLUTION PRODUCTS FROM BIOACTIVE GLASS-CERAMIC SCAFFOLDS 45S5.2B POSITIVELY MODULATE THE IN VITRO CELL RESPONSE UNDER CONDITIONS OF HYPERGLYCEMIA

Haro Durand LA, Baldi A, Fanovich MA, Boccaccini AR, Gorustovich A.
Laboratorio de Patología y Farmacología Molecular IByME-CONICET, División Cerámicos INTEMA-UNMdP-CONICET, InstituteofBiomaterials, Universityof Erlangen-Nuremberg y Grupo Interdisciplinario en Materiales IESIING-UCASAL INTECIN UBA-CONICET.E-mail: harodurand.luis@gmail.com

One of the main complications of diabetes mellitus (DM) is the failure in the process of tissue repair due to cellular dysfunction as a result of hyperglycemia. Under hyperglycemic conditions, the main cells involved in restoring the functionality and integrity of tissues

are unable to migrate, proliferate, and secrete growth factors and components of the extracellular matrix, all of which is favored by altered mechanisms of glycation and oxidation. This alteration invariably contributes to failures in repair processes. Thus, it is of biomedical interest to study different therapeutic strategies to optimize the repair and/or regeneration of tissues under hyperglycemic conditions. These strategies may include the use of different biomaterials in the form of three-dimensional porous matrices, known as scaffolds. The ideal design of these scaffolds should provide temporary biocompatible mechanical support and positively modulate the cellular response. Since it has been established that boron (B) plays a role in angiogenesis and tissue repair, it can be expected that the controlled and localized release of B ions from the bioactive glass-ceramicscaffolds could represent a promising alternative therapy in regenerative medicine of vascularized tissues in patients with DM. The aim of this work was to study the *in vitro* cellular response of ionic dissolution products from bioactive scaffolds manufactured from the controlled crystallization of a 45S5 glass (% w/w composition: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6%P_2O_5) addedwith 2% of B_2O_3 (45S5.2B), in primary cultures of fibroblasts (HDF) and endothelial cells (HUVECs) grown in hyperglycemic conditions (30 mM D-glucose). The results demonstrated, for the first time, that the ionic dissolution products released from the 45S5.2B bioactive glass-ceramic scaffolds positively modulate the *in vitro* cellular response of fibroblasts and endothelial cells grown under hyperglycemic conditions. This was corroborated by an increased proliferative and migratory response, greater ability to form tubules *in vitro* and an increase in the secretion of growth factors. These findings may be relevant in vascularized tissue engineering since scaffolds obtained from the 45S5.2B bioactive glass could act as inorganic agents that positively modulate the cellular response and favor processes of tissue repair and/or regeneration in patients with DM.

A93
MESOPOROUS TITANIA COATING: DETERMINATION OF ITS PHYSICOCHEMICAL PROPERTIES AND YEAST BEHAVIOR

Morcillo MF, Tuttolomondo MV, Catalano PN, Bellino MG, Soler-Illia GJAA, Desimone MF.
Cátedra de Química Analítica Instrumental (Departamento de Química Analítica y Fisicoquímica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina), Junín 954, 3° piso, CABA, Argentina. IQUIMEFA-CONICET, Junín 954 (1113) CABA, Argentina.E-mail: marinafmorcillo@gmail.com

We have synthesized titania mesoporous films using titanium (IV) chloride as sol-gel precursor and surfactants from Pluronics® (Pluronic F-127, POE-POP-POE) and Brij ™ series (B96 y B58, alquil-POE) that act like molds for pore size and distribution. Nanotopography is known to be key for adhesion and cellular growth. In this experiment, we demonstrate that nanotopography is also determinant for proliferation of eukaryotic microorganisms such as *Candida albicans* (ATCC 10231). We cultivated the microorganism in the presence of the films, the films derivatized with APTES ((3-Aminopropyl)triethoxysilane) and the films derivatized with APTES and then doped with $CuCl_2$ (a known fungicide). Results show that mesoporous films prepared with Brij-96 presented the best outcome in regards of controlling cellular proliferation (up to a 75% inhibition of development in the Brij-96 derivatized with APTES-$CuCl_2$). We also characterized the mesoporous films by performing a scratch essay, which determines damage resistance of the material, and a contact angle assay. Results showed that every film tested can resist up to 40N without tearing and the contact angle that determines superficial hydrophilicity, allowed us to establish that the coating made with Brij-96 is the least hydrophilic coating of all. This is in accordance with minor cellular proliferation results using this surface. We observed our films through scanning electron microscopy (SEM) and demonstrate that films derivatized with APTES or with APTES-$CuCl_2$ did not change the original nanotopography of the coating. Lastly, we did an EDS (Energy-dispersive X-ray spectroscopy) essay to ratify the presence of Ti in all of the films, C and N in the ones derivatized with APTES, and C, N and Cu in the ones derivatized with APTES-$CuCl_2$.

A94
CELL ADHESION AND BIOCOMPATIBILITY OF PVA SPONGES CROSSLINKED WITH HEXAMETHYLENE DIISOCYANATE FOR REGENERATION OF BONE TISSUE

Perna O, Alvarez Echazu MI, Olivietti CE, Garcia Cuniglio, SB, Desimone MF, Alvarez GS.
Facultad de Farmacia y Bioquímica (FFyB-UBA), IQUIMEFA-CONICET. E-mail: orianaperna17@gmail.com

PVA sponges crosslinked with hexamethylene diisocyanate (HDI) were developed to obtain hydrophilic and slowly-degradable scaffolds for biomedical purposes with good biocompatibility and better mechanical properties. The obtained PVA-crosslinked with different concentrations of HDI was characterized by IR spectroscopy where it was possible to observe a reduction in the peaks intensity at 3300 cm^{-1} corresponding to O–H stretching, indicating the effective reaction of HDI with PVA hydroxyl groups. It was also possible to determine that the NCO stretching peak at 2280^{-1} disappeared after the reaction with PVA except for the highest concentration. Also we observed an increment in the signal at 1750 cm^{-1} corresponding to free C=O stretching as the mobility of molecular chains was restrained by the reaction between –OH and –NCO, which prevents the formation of hydrogen bonds. The materials were also observed by electron microscopy and the increment in nitrogen content due to HDI was measured by EDX. The swelling radio and degradation was evaluated with the intention to demonstrate the long-term physical integrity of the material. PVA sponges swelling ratio was 400% while for HDI crosslinked sponges this value was modified to 150%. Materials with the highest percentage of HDI did not show significant degradation after three weeks of incubation. Furthermore, an osteoblastic cell line 3T3 E1 was used to evaluate cell adhesion to the materials and their cytocompatibility. According to these studies, PVA is an attractive

material for these applications because of its biocompatibility and low protein adsorption properties which result in low cell adhesion compared with other hydrogels. However, this fact may complicate its application in regenerative medicine as cell scaffold for osteoblast colonization. For this reason, PVA- crosslinked hydrogels were incubated with collagen I and IV to increase cell adhesion to the materials. It was possible to observe that the system was not toxic for the cells and a significant increment between 4 and 5 times with collagen IV coating extracted from placenta was obtained but not such results were obtained for collagen type I.

BEHAVIOUR AND NEUROSCIENCES

A95
CHRONIC EXPOSURE DURING ADULTHOOD TO A GLYPHOSATE BASED HERBICIDE ALTERS THE EXPRESSION OF HORMONAL RECEPTORS IN HYPOTHALAMIC NUCLEI INVOLVED IN THE RAT ESTROUS CYCLE REGULATION

Canesini G, Stoker C, Lazzarino GP, Rossetti MF, Schumacher R, Gastiazoro MP, Durando ML, Varayoud JG, Ramos JG.
Instituto de Salud y Ambiente del Litoral (ISAL; UNL-CONICET); Facultad de Bioquímica y Ciencias Biológicas –Universidad Nacional del Litoral. E-mail: gcanesini@fbcb.unl.edu.ar

Glyphosate-based herbicides (GBHs) are extensively used to control weeds on both cropland and non-cropland areas. In Argentina, glyphosate is the most commonly used herbicide, with around 200–260 million liters applied every year. It has been reported that GBHs may act as an endocrine disruptor. However, the possible consequences of this pesticide exposure on fertility and specifically on hypothalamic function remains poorly understood. In the normal cycling rat, an increase in luteinizing hormone (LH) occurs, triggered by the neurosecretion of the LH-releasing hormone (LHRH), also known as gonadotropin-releasing hormone (GnRH). In rats, the majority of LHRH-secreting neurons are located in the preoptic area of the hypothalamus. Activation of steroid receptors in specific hypothalamic regions like the anteroventral periventricular nucleus (AvPv) and the arcuate nucleus (Arc) is also necessary to achieve a normal LH surge. The aim of this study was to describe the effects of chronic exposure during adult life at a dose of GBH close to the reference dose, on the estrous cycle of the rat and on the expression of key proteins of the hypothalamic nuclei involved in its regulation. Adult rats were exposed for 3 months in the pellet chow to 2 mg/kg/day of GBH (GBH group- GG) or control diet (pellet chow with saline solution – control group- CG). The estrous cycle was monitored by vaginal cytology for two weeks and finally the animals were sacrificed approximately at day 240 of age in diestrous stage. The brains were dissected, fixed and included in paraffin, and coronal sections of 5µm were obtained by microtomy. The estrogen receptor alpha (ERα) and progesterone receptor (PR) were assessed by inmunohistochemistry (IHC) in hypothalamic AvPv, Arc and medial preoptic (Mpo) nuclei. The estrous cycle of GG animals was affected since they showed a low percentage of time in the proestrous-estrous stages when they were compared to CG. ERα expression was significantly lower in GG respect to CG, in the three nuclei evaluated. Moreover PR expression was lower in AvPv nucleus of GG rats and significantly higher in Mpo and Arc nuclei in GG respect to CG. These results show that a dose of GBH considered safe, administered chronically through diet in adult life, alters the rat´s cyclicity and modifies the expression of key brain proteins involved in its regulation. Furthermore, these changes may provide new evidence on the possible effects produced by glyphosate at hypothalamic level that could affect fertility.

A96
LONG-TERM MEMORY IMPAIRMENTS FOLLOWING SUCROSE EXPOSURE IN JUVENILE VERSUS ADULT RATS

Kruse MS, Vadillo MJ, Rey M, Coirini H.
Instituto de Biología y Medicina Experimental-CONICET. E-mail:sol.kruse@conicet.gov.ar

We previously described that excessive consumption of sucrose in early stages of development has deleterious neurobiological and behavioral effects in adulthood. In the present study, we evaluated whether the short-term, long-term and consolidated memory are also compromised by early sucrose consumption. 25 days after unlimited sucrose access during youth or adulthood, animals were briefly exposed to two identical objects in a 75 cm x 55 cm arena, and memory was evaluated in consecutive test trials by exchanging one of the familiar objects for a novel one. Control and sucrose-exposed rats spent the same time exploring the two objects during the sample session T1. However, when the four groups were exposed 2 h later to a copy of the objects presented in the sample session and a novel object (short-term memory, T2), control animals or animals that drank sucrose during the adulthood, but not during youth, exhibited a clear preference to explore the novel object as shown by the significant interaction between age and treatment (two-way ANOVA; $F(1,28) = 4.208$, $P = 0.0497$). Control rats and rats treated in adulthood continued to exhibit a preference to explore the novel object over the familiar one 24 h (long-term memory, T3) and 7 days after the training session (consolidated memory, T4). In contrast, rats exposed to sucrose during youth were again unable to distinguish between the novel and the familiar objects. Two-way ANOVA indicated a significant age and treatment interaction, on the capacity to discriminate the novel from the familiar object (T3; $F(1,32) = 11.908$, $P = 0.0015$ and T4; $F(2,21) = 4.932$, $P = 0.0370$). Altogether these results suggest that unlimited sucrose exposure during the child-adolescence produced long-term alterations of the hippocampus, prefrontal and perirhinal cortex and/or their connections.

A97
NATIVE PLANTS AND NEUROPROTECTIVE EFFECTS IN DIABETES MELLITUS

Rey M[1], Kruse MS[1], Magrini Huamán RN[2,3], Tapia AA[2], Feresin GE[2], Coirini H[1,3,4]
[1]Lab Neurobiología, IBYME-CONICET,CABA; [2]Inst de Biotec., Fac de Ing, UNSJ, San Juan,[3]Fac de Cs. Méd., UCCuyo, San Juan; [4]Dept Bioq.Hum.-FMED-UBA, CABA. E-mail: mariana.rey@ibyme.conicet.gov.ar

In Cuyo region (Argentine) *Tessaria absinthioides* (Ta) and *Oxalis erythrorhiza* (Oe) are popularly consumed to regulate glucose (Glu) and cholesterol (Chol) levels, lacking of scientific support. Several works indicate that 85% of world population uses "medicinal plants" to treat its health. Diabetes mellitus patients have chronic hyperglycemia, oxidative stress state and can also develop neurodegenerative changes. This is a problem for health systems, thus the search of alternative therapies remains in force. Previously, we had shown effects of Ta and Oe decoctions (10% W/V) on metabolism and LXRs expression in hypothalamus (HT). In this work, adult male rats (SD), controls (C, i.p.veh.) or diabetics (D, i.p. STZ 30 mg/Kg) drink decoctions (10% W/V) of Ta (CTa/DTa), Oe (COe/DOe) or water (CW/DW) by 4 weeks. Glu, Chol and triglycerides (Tg) levels were measured on blood samples by commercial kits. Neural NOS (nNOS) and Neurofilaments 200 (NF200, 3 different bands) expression levels were studied in HT by WB. Compared to CW, DW, DOe and DTa had higher Glu (350%, 200% and 200% resp.; $p<0.05$) and Tg (200%, 300% and 200% resp.; $p<0.05$) levels. But, Glu in DTa and DOe was lower than DW (both 150%; $p<0.05$). No changes were observed in Chol among groups. All NF200 bands were higher in DW, COe and CTa than CW (28%; 14% and 19% resp.; $p<0.05$). However, the value of NF200 in DTa were lower than DW (25%; $p<0.05$) and closer to CW. In DW and COe the nNOS were higher than in CW (43% and 70% resp.; $p<0.05$), however the decocctions had not effect on D. Results suggest that these plants could have metabolic and neuroprotective effects. A more extended treatment will be necessary to propose Oe and Ta like new therapeutic tools (PIP0243,PIO-SECITI2250,CICITCA UNSJ,CONICET).

A98
EFFECT OF THE PROINFLAMMATORY MICROENVIRONMENT ON HUMAN DOPAMINERGIC PRECURSORS SURVIVAL AND DIFFERENTIATION

Wenker S (1), Gradaschi V(1), Farias M (1), Garcia C (1), Ferrari C (2), Zeng X (3), PitossiF(1).
(1) Foundation Leloir Institute –IIBBA-CONICET. (2) Italian Hospital. (3) Buck Institute For Research On Aging.
E-mail: swenker@leloir.org.ar

Parkinson ´s Disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons of the substantia nigra pars compacta. Successful transplantation of dopamine-producing cells into the striatum has been shown in animal models and clinical trials. However, beneficial effects are constrained because of the remaining low number of DA neurons grafted. This could be due to host inflammatory response, among other factors. Few reports have addressed the issue of inflammatory response effect on the survival and differentiation of the transplanted cells. We studied the host primary response related to the graft of human DA precursors (DA14) *in vivo* and the impact of proinflammatory factors on the viability and differentiation process of DA14 cells *in vitro*.Our *in vivo* resultsshowed a significant increase ($P<0.05$) of host-MHCII and GFAP positive cells associated to the human graft after 15 days post-transplant of DA14 cells into immunosuppressed rats. To study the effect of microglial activation, BV2 microglial cells were treated with LPS, and nitrite and TNF alpha production were determined. DA14 cells were exposed to conditioned media (CM) from basal and activated BV2 cells during 4 days and immunofluorescence (IF) for Tyrosine hydroxylase (TH) (DA neuronal marker) was performed at DA28 stage. Results from TH cell counting revealed that DA14 incubated with CM from activated microglia significantly decreased the number of DA neurons at DA28 ($p<0.05$). In order to study the short term effect of inflammatory environment, DA14 cells were then exposed toCM from basal and activated BV2 cells during 4 days. After this treatment differentiation and survival assays were performed. TH cell counting suggested that CM from activated microglia during 4 days significantly decreased the number of DA cells ($p<0.05$). During this period of time, Hoechst staining showedthat the inflammatory factors from activated microglia significantly increasedcell death ($p<0.05$). DA cell morphology analysis was performed to evaluate the differentiation process. Decrease in the percentage of neuronal morphology-like cells was convoyed by neurite length alterations after 4 days with CM activated microglia. In this proinflammatory context, we also observed a negative modulation of two transcription factors involved in dopaminergic differentiation: Foxa-2 and Nurr-1. Altogether our findings suggest that, microglial cells could play a fundamental role in the survival and differentiation of DA precursors. The cellular and molecular mechanisms involved in these processes should be considered and deeply analysed to improve cell replacement therapy.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

REPRODUCTION III

A99
SPERM MATURATION DEFECTS IN CRISP1/CRISP4 DOUBLE KNOCK OUT MICE

Carvajal G, Visacovsky N, Weigel Muñoz M, Cuasnicu P S.
[1]Instituto de Biología y Medicina Experimental (IBYME-CONICET), CABA, Argentina.E-mail: guillec6@hotmail.com

To become fertilization competent, sperm must undergo both epididymal maturation in the male tract and capacitation in the female tract. During epididymal maturation, sperm undergo a series of biochemical and physiological changes that involve the incorporation of new molecules synthetized by the epididymal epithelium as well as post-translational modifications. CRISP1 and CRISP4 are two members of the Cysteine Rich Secretory Protein (CRISP) family that associate with the sperm surface during epididymal transit and are key mediators of fertilization. Double KO males (DKO) for these proteins are subfertile and exhibit sperm fertilizing defects and both an immature epididymal epithelium and abnormal luminal pH. To gain insights into the mechanisms underlying this phenotype, in the present work, we analyzed different sperm parameters associated with epididymal maturation. Results showed that DKO animals presented altered cauda sperm motility and a higher percentage of sperm with cytoplasmic droplets. Using Fluo4-AM and flow cytometry, we observed that while cauda epididymal sperm presented no changes in basal intracellular calcium concentrations compared to controls, they exhibited different calcium levels after calcium ionophore exposure, supporting changes in calcium homeostasis in the DKO cells. Moreover, flow cytometry analysis using merocyanine dye and DiSBAC2(3) probe to evaluate membrane fluidity and membrane potential, respectively, showed lower values for both parameters in DKO sperm compared to controls. Altogether, these results reveal defects in the maturation state of DKO caudal sperm supporting the relevance of CRISP1 and CRISP4 for sperm epididymal maturation.

A100
EFFECT OF GnRH POST INSEMINATION ON LUTEAL FUNTION IN SHEEP

Fernández J[1,4], Bruno Galarraga M[1,3], Soto A[2], de la Sota R[2,4], Cueto M[1], Gibbons A[1], Lacau I[3,4].
[1]INTA EEA Bariloche.[2]Fac. Cs Vet. UNLP.[3]IBYME CONICET. [4]CONICET. E-mail: fernandez.jimena@inta.gob.ar

We evaluated the effect of the administration of gonadotropin-releasing hormone (GnRH) at Day 4 post timed artificial insemination (TAI) on the formation of accessory corpora lutea (accCL) and the production of luteinizing hormone (LH) and progesterone (P_4) in sheep. During the breeding season, a total of 24 adult Merino ewes were randomly assigned to two groups on Day 4 post TAI: GnRHGroup (n= 12; 4 µg of Buserelin, i.m., Receptal®, Intervet, Arg.) and Control Group (n= 12; 1 ml of saline solution, i.m.). Laparoscopic observation of the ovaries at Days 4, 10 and 21 post TAI was performed to determine the presence of ovulatory CL and accCL. Serum P_4 concentration was assessed by chemiluminescence on days 4, 7, 10, 13, 17, 21, 28 and 35 post TAI. Serum LH concentration was assessed by RIA 2-6 h post treatment. The number of accCL and LH concentration was analyzed by ANOVA and P_4 concentration by a generalized linear model with time-repeated measurements. Statistical significance was accepted from $P<0.05$. The presence of an accCL was only observed in the GnRH Group ($P<0.05$).GnRH administration did not increase serum P_4 concentration (5.8±0.4 ng/ml) compared to the Control group (5.3±0.3 ng/ml) ($P>0.05$). Moreover, GnRH treated ewes had similar concentration of P_4, whatever they developed or not an accCL ($P>0.05$). GnRH administration increased serum LH concentration about 2-6 h after treatment compared to the Control group (10.6±1.2 ng/ml and 3.1±1.1 ng/ml, respectively; $P <0.05$). The increase of LH concentration was observed only in the ewes that generated acc CL ($P<0.05$). Administration of GnRH at Day 4 post TAI increased serum LH concentration and induced the formation of accCL. However, serum P_4concentration did not increase.

A101
FUNCTIONAL STUDIES TO EVALUATE BOVINE OOCYTE VITRIFICATION

Gutnisky, C[1,2],Morado, S[1], Alvarez, G[1,2], Cetica, P[1,2]
Universidad de Buenos Aires, Facultad de Cs. Veterinarias. [1]INITRA (UBA), [2]INPA (UBA-CONICET).E-mail: cgutnisky@gmail.com

Previous studies using a minimum volume vitrification system (Cryotech® method) for bovine oocytes showed an excellent recovery of their viability and normal morphology after vitrification/warming process. Our main aim was to evaluate the effect of the vitrification/warming by this system on functional parameters evaluating metaphase II recovery, in vitro fertilization (IVF), parthenogenetic activation and embryo development. Partially denuded matured oocytes were vitrified and warmed using the Cryotech® vitrification method. To study the time of recovery of the metaphasic plate II, vitrified/warmed oocytes were incubated for 2, 3 and 4 h. After incubation, oocytes were stained with fluorochrome Hoechst 33342 to establish the time point at which the majority of the metaphasic plates appear. Three groups of in vitro matured oocytes were fertilized: oocytes surrounded by cumulus cells (IVF control), partially denuded oocytes (control) and vitrified/warmed oocytes (treatment). IVF was performed using frozen–thawed semen from a Holstein bull of proven fertility. Semen was thawed at 37ºC in mSOF. The proportion of cleaved oocytes was determined by evaluating the number of embryos that presented two or more blastomeres. In vitro embryo development was performed in IVC-mSOF, under mineral oil. The proportion of blastocysts produced was determined at day 7 following insemination. Parthenogenetic

activation was performed in TALP plus ionomycin for 4 minutes and then oocytes were incubated in IVF-mSOF with 6-dimethylamino purine for 3 h, before being incubated for 21 h inIVF-mSOF. Metaphase II configuration recovered between 3 and 4 h after warming (65.4 %), being 2 h-incubation post warming insufficient for recovery (P<0.05). Significant differences between the three groups described above were observed in the cleavage and blastocyst rates, observing a decrease in vitrified/warmed oocytes (cleavage rates: IVF control 78.60 %, control 56.98 %, vitrified/warmed oocytes 27.40 %; blastocyst rates: IVF control 26.40 %, control 9.80 %, vitrified-warmed oocytes 0 % P<0.05). On the other hand, parthenogenetic activation showed no difference between groups (control: 54%, vitrified/warmed oocytes: 52%). Therefore, vitrification adversely affects cleavage rate and subsequent embryo development in sperm-activated bovineoocytes, however, in parthenogenetically activated oocytesthe cleavage rate was not affected.

A102
EFFECT OF IONOMYCIN ON THE *IN VITRO* FERTILIZATION OF PIGS

Luchetti CG[1,2], Lorenzo MS [1,2], Lombardo DM [2].
[1]CONICET [2]Universidad de Buenos Aires. Facultad de Ciencias Veterinarias. Instituto de Investigación y Tecnología en Reproducción Animal (INITRA). Cátedra de Histología y Embriología. Chorroarin 280, CABA, Argentina.
E-mail: caroluchetti@gmail.com

Given the relevance of porcine embryo production for commercial purposes or for biomedical studies, many research laboratories have focused on the optimization of the *in vitro* fertilization (IVF).To improve its efficiency, the effects of ionomycin (Io) on IVF were studied. Porcine COC obtained by follicular aspiration from slaughterhouse ovaries were *in vitro* matured in TCM-199 + porcine follicular fluid (10%), FGF (3ng / mL) and FSH (30 ug / mL) during 44-47 h. Then, matured oocytes were denuded with hyaluronidase and treated with 5 uM ionomycin for 4 min and washed in TALP-H medium four times (group Io+), or without it: control (group C). Immediately, oocytes were subjected to IVF (coincubated 30 min with fresh boar semen $1x10^6$ sperm / mL in modified BO medium). The presumptive zygotes were *in vitro* cultured in 50 ul drops of SOF medium at 39° C with 5% CO_2, 5% O_2 and 100% humidity. After 20 h from the beginning of the IVF, embryos were fixed in paraformaldehyde 4% during 30 min, washed and stored in PBS-PVA until use. To assess fertilization parameters presumptive zygotes were stained with Hoëchst and mounted for evaluation. The fertilization parameters evaluated were: penetration (oocytes penetrated/ inseminated), masculine pronuclear formation (MPN=oocytes containing MPN/penetrated), monospermy (oocytes containing only one sperm head or one male pronucleus/ penetrated), and efficiency of fertilization (monospermic oocytes/inseminated). Data were analyzed by Fisher's exact test considering significant P <0.05 (n control=99; n Io+=89). Io+ showed significantly higher penetration rate than control (P<0,001), MPN (P<0.05), and efficiency of fertilization (P<0.05), whereas monospermy showed no differences. These results complete our previous study of embryo development under similar conditions where Io+ increase the cleavage rate, and it remained to be determined whether this increase is due to a higher efficiency of fertilization or a parthenogenetic activation. We conclude that the treatment of porcine oocytes with ionomycin is a tool to contribute to the IVF optimization.

A103
ACTIONS OF MELATONIN IN HAMSTER TESTICULAR PERITUBULAR CELLS

Riviere E (1), Matzkin ME (1,2), Cigorraga SB (3), Pellizzari EH (3), Calandra RS (1), Mayerhofer A (4), Rossi SP(1,2), Frungieri MB (1,5).
(1) Instituto de Biología y Medicina Experimental, CONICET, Buenos Aires, Argentina. (2) Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina. (3) Centro de Investigaciones Endocrinológicas, CONICET, Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina. (4) Biomedical Center Munich (BMC), Cell Biology, Anatomy III, Ludwig-Maximilian-University (LMU), Planegg, Germany. (5) Ciclo Básico Común, Universidad de Buenos Aires, Buenos Aires, Argentina. E-mail: mfrungieri@dna.uba.ar

Peritubular cells, surrounding the seminiferous tubules of the testis, are well known for their implication on tubular contractility and sperm transport. Together with Sertoli cells, they form the cellular boundary of the spermatogonial stem cell (SSC) niche. However little is known about the contribution of peritubular cells to the SSCs and its niche, mainly as secretome cells. The objectives of this study were: i) to isolate and characterize immature hamster testicular peritubular cells (haTPCs), and ii) to investigate the potential actions of melatonin on the contractile and synthesis activities of haTPCs. haTPCs were isolated from testes of immature Syrian hamsters (21-23 days old) by successive enzymatic digestions (trypsin and collagenase) and subsequently characterized by immunocytochemistry. haTPCs showed positive staining for a peritubular cell marker (α-smooth muscle actin, α-SMA) but negative staining for Sertoli cell markers (SOX9 and anti-Müllerian hormone, AMH) and a germ cell marker (VASA). haTPCs express the melatonin receptors subtype MT1 and MT2. Incubation of haTPCs with 1μM melatoninsignificantly increased mRNA and/or protein expression of the contractile markersα-SMA and calponin.mRNA expression of the glial cell line-derived neurotrophic factor (GDNF), a factor involved in the maintenance of the SSCs self-renewal, was also upregulated by melatonin (*P*<0.05). In addition, 1 μM melatonin increased mRNA and protein expression of cyclooxygenase 2 (COX2), key enzyme in prostaglandin (PG) synthesis, as well as the production of PGD2 (*P*<0.05). haTPCs and hamster Sertoli cells express PGD2 receptors (DP receptors). Moreover, glucose uptake and lactate production in Sertoli cells purified from photoperiodically regressed adult hamsters showed a statistically significant increase in the presence of 1μM PGD2. Therefore primary cultures of peritubular cells are an important tool for studying not only the structural role of these cells in the testis but also for understanding their function in the homeostasis of the testicular environment, i.e. regulation of the SSCs and its niche. In this study, we specially demonstrated a regulatory role exerted locally by melatonin in haTPCs.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

A104
SPHINGOLIPID CERAMIDE-1-PHOSPHATE (C1P) IN OVARY AS A PROMISING STRATEGY FOR DELAYING OVARIAN AGING

Scotti L[1], Pascuali N[1], Oubiña G[1], Mena A[2], Velazquez C[1], Negri Montes L[1], de Zúñiga I[3], Negrotto S[2], Abramovich D[1] and Parborell F[1].
1) Instituto de Biología y Medicina Experimental (IByME-CONICET), 2) Instituto de Medicina Experimental (IMEX, CONICET-ANM-BA), 3) PREGNA Medicina Reproductiva. E-mail: leopoldinascotti@gmail.com

As many women postpone childbearing over 38 years a large portion of aged female becomes infertile. Ovarian aging is dominated by the progressive loss of primordial follicles and a decline in oocyte quality. An active blood supply via ovarian angiogenesis seems to be essential for the induction of oocytes with good quality. Activation of ovarian angiogenesis has emerged as a new strategy to halt age-related decline of ovarian response. Endothelial progenitor cells (EPCs) are a population of bone marrow-derived mononuclear cells thought to participate in endothelial repair. Since the bioactive sphingolipid C1P is an important proangiogenic factor, our aim was to: 1) evaluated the local effect of C1P on EPCs mobilization and on ovarian angiogenesis in aged female mice and 2) evaluated the angiogenic potencial of follicular fluid (FF) from aged patients in the presence of C1P in endothelial cells (ECs). Aged female mice (26-31 weeks) received C1P (10µl/ovary; 50 µM) under the bursa of the ovaries. The animals were sacrificed 2 and 7 days later, and the ovaries were isolated. Young mice (6-9 weeks) were used as control. Prior to surgery and sacrifice, peripheral blood (PB) was extracted from the maxillary cavity to evaluate by flow cytometry EPCs mobilization (VE-Cadherin+, Sca-1+). Histological and immunohistochemical analysis (VW: endothelial cell marker, α-SMA periendothelial cell marker), and Western blot (VEGF-A) were performed. ECs were incubated in the presence of FF from aged patients (38-42 years) and young patients (<38 years). C1P (10 µM) was added in FF. ECs migration was evaluated by wound healing assay and VEFG-A by Western blot. EPCs were detected in PB 7 days post administration of C1P in ovaries from aged mice. The % of primordial and antral follicles in aged mice was lower than in young mice ($p<0.05$), while atretic follicles was higher in aged mice than in young mice ($p<0.001$). The local administration of C1P prevented the loss of primordial follicles in ovaries from aged mice at 7 days ($p<0.05$), and increased the % of antral follicles and decrease the % of atretic follicles ($p<0.001$) in both times of treatment with C1P ($p<0.05$). The endothelial and periendothelial cell area increased in ovaries from aged mice treated with C1P at both time ($p<0.05$). VEGF-A levels increased in ovaries from aged mice at 7 days ($p<0.05$). ECs migration and VEGF-A expression decreased in cells incubated with FF from aged patients compared to young patients ($p<0.001$). The presence of C1P in FF from aged patients increased the ECs migration ($p<0.05$), but had no effect on VEGF-A expression. C1P administration in aged mice improves the ovarian angiogenesis, possibly by mobilization of EPCs to this organ. These results may have potential clinical implications in the treatment of age-related infertility.

BIOCELL 43 (suppl. 3), 2019
ISSN 0327- 9545
ISSN 1667-5746 (online version)

AUTHOR INDEX

M

N

O

P

Q

R

S

T

U

V

W

Y

Z