ABSTRACT BOOK

JOINT MEETING

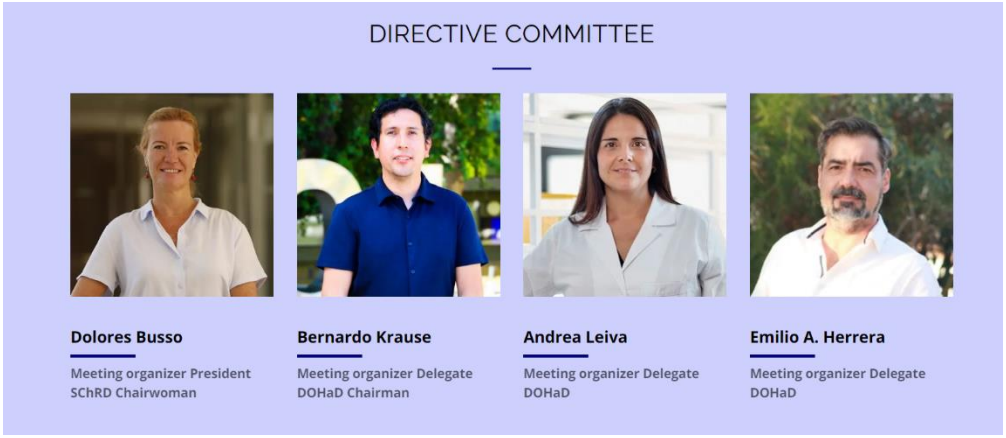
V Meeting of the Latin American Regional Society for Developmental Origins of Health and Disease (LA-DOHaD)

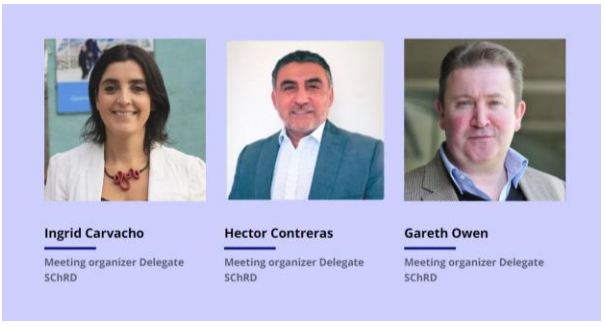
&

XXXIV Meeting Chilean Society of Reproduction and Development

Valdivia, Chile 2023

V Meeting Latin American Regional Society for Developmental Origins of Health and Disease (DOHaD) and XXXIV Meeting Chilean Society of Reproduction and Development





Organizing Committee: Dolores Busso – Bernardo Krause – Emilio Herrera – Monika Greiner – Andrea Leiva – Ingrid Carvacho – Maritza Garrido – Francisca Salas

Scientific Committee: Emilio Herrera - Gareth Owen – Héctor Contreras – Alfonso Paredes – Paola Casanello - Alejandro González-Candia - Macarena Lépez

Local Committee: Natalia Mendez – Alfredo Ramirez

Index

Plenary lectures:

“Epigenetic mechanisms in perinatal programming: a new perspective for DOHad”

Torsten Plösch
University Medical Center Groningen (UMCG), Groningen, The Netherlands

Chair: Andrea Leiva

In human development, the first 1000 days of life are known to prime the development of our later health. Many studies have shown that the in utero environment and early postnatal phase have long-lasting consequences for the vulnerability to develop chronic diseases at adult age. Stimuli identified in humans include external factors such as maternal or neonatal malnutrition, stress, and smoking, but also internal factors as placental dysfunction or maternal infection. These early factors are known to change the epigenome and, hence, modify the phenotype in the long run.

To address the relationship between early development and later disease, we combine animal studies and human cohorts. I will here highlight our recent studies focusing on the long-term consequences of placental dysfunction and preeclampsia in mice, using a novel model using a combination of anti-angiogenic factors (sFlt1) and inflammation (LPS). These studies specifically show long-term metabolic consequences for the offspring. Moreover, I will introduce some of our human studies in preterm or growth-restricted infants, especially with respect to neurological outcomes. The main topic will be the mediating role of epigenetics and sex-specific phenotypes.

“Early Origins of Obesity: evidence from the Chilean CIAPEC-Cohorts”

Camila Corvalán
University of Chile, Institute of Nutrition and Food Technology (INTA), Center for Research in Food Environments and Obesity

Chair: Gareth Owen

Background: in Chile, like in most countries worldwide, unhealthy diets and nutrition are the main cause of death and disability. Thus, understanding how early life exposures are associated with these conditions can contribute to improving public health outcomes.

Methods: over the past 15y the Center for Food Environment and Obesity Research (CIAPEC) has conducted longitudinal studies in key periods of early life (pregnancy; infancy; preschoolers & adolescents) to better understand the role of nutritional exposures on the early development of NCDs. Participants have been recruited from the south-East area of Santiago, capital of Chile, and represent low-middle income Chileans. In each of the cohorts (~1,000 participants), we have obtained repeated anthropometric, body composition, sociodemographic, dietary, and mental health information as well as biological (blood, urine, saliva, fecal, DNA) samples to characterize metabolic, inflammatory, hormonal status and genetic background. We have also characterized the food environment surrounding these people, including food availability, food composition, food advertising, and food prices, among other aspects.

Results: our studies show that consumption of ultra-processed foods (i.e., unhealthy foods) is highly prevalent in our cohorts, while the consumption of fresh food is infrequent, resulting in a high prevalence of obesity and metabolic diseases such as metabolic syndrome, insulin resistance, and lipid disorders. We have also observed that diet and nutrition during pregnancy, infancy, and puberty are associated with growth and maturation as well as the risk of obesity and associated metabolic conditions. Finally, we have explored how clinical and food environment interventions can contribute to improve diet and nutrition in these critical periods, identifying some areas for progress.

Conclusions: longitudinal studies of low-income mothers and children in Chile confirm the relevance of early life periods for later growth and development; implementing individual and environmental preventive actions oriented to these groups is urgently needed for improving nutrition and health.

Keywords: Developmental Origins of Obesity, CIAPEC Longitudinal Studies, Nutritional Epidemiology.

Financing: This research has been funded by several national and international agencies, including ANID, NIH, IDRC, and Bloomberg Philanthropies

KEYNOTE SPEAKERS' SYMPOSIUM: Nutrition and Metabolism in Development and Beyond

Chair: Bernardo Krause

"Exposure to maternal diabetes in pregnancy - new insights from mouse models"

Claudia Kappen
Pennington Biomedical Research Center, United States

Background: Maternal diabetes during pregnancy raises the risk for birth defects, particularly heart and neural tube defects. Offspring are also predisposed to metabolic and cardiovascular disease as adults. Since better glycemic control is associated with fewer adverse outcomes, it is widely believed that excess glucose is the culprit in "fuel-mediated teratogenesis".

Methods: We employed mouse models, transcriptome profiling by RNA-seq, single-cell RNA sequencing, bioinformatics, lipidomics and metabolomics, as well as spatial imaging by MALDI-IMS and histology.

Results: Gene expression profiling revealed that exposure to diabetic conditions is detectable as early as E7.5 days of development. Embryos in diabetic conditions experience nutritional stress, demonstrable at the level of individual cells. In these conditions, mesodermal cells exhibit impaired migration out of the primitive streak. Unexpectedly, administration of glucose can alleviate the adverse exposure, indicating that glucose supply to the embryo is deficient. Untargeted metabolomics provided supporting evidence. We also detected retention of lipids in the visceral yolk sac, confirmed by LC-MS and MALDI imaging.

Conclusions: Our work in the past decade has established that exposure to diabetes affects embryos much earlier than previously recognized. Contrary to the prevailing dogma of nutrient overload, our recent studies provide multiple lines of evidence that embryos in diabetic pregnancies experience nutrient shortage. Imaging data demonstrate nutrient retention in the visceral yolk sac, the source of nutrients before the embryonic circulatory system is connected to the placenta. Our nutrient deficit hypothesis not only can explain the broad spectrum of birth defects in diabetic embryopathy, but also the well-documented intrauterine growth reduction in rodent diabetic pregnancies. We posit that re-evaluation of prior findings required in light of our results will prompt a major paradigm change in the field, with implications for the care of women with diabetes, and for prevention of birth defects in their offspring.

Funding: Grants R01-HD085017 (to J.M.S) and R01-HD087283 (to C.K.), and Research Center grants P30-DK072476 and P30-GM118430 to PBRC from the US National Institutes of Health.

"Early-life Iron Deficiency: Neurodevelopmental Consequences and Adjunctive Treatment with Choline Supplementation"

Phu V Tran, PhD
University of Minnesota, United States

Early-life iron deficiency (ID) affects ~40% of all pregnant women worldwide and causes long-term impairments in cognition and socio-emotional behaviors in offspring in spite of iron treatment. The persistent abnormalities constitute a significant cost to individual and society in terms of educational attainment. The underlying pathobiology for the persistent abnormalities has been ascribed to impaired neural development and function (e.g., energy metabolism, dendritogenesis, and synaptogenesis) and widespread gene dysregulation, both carried forward into adulthood. Despite this knowledge, major gaps remain, including the lack of a mechanistic understanding underlying the long-term changes.

The presentation will discuss evidence linking iron-dependent epigenetic mechanisms, gene regulation, and changes in enrichment of neural cell types. Given the insufficiency of iron treatment alone in correcting ID-impaired neurodevelopment, the beneficial effects of prenatal choline supplementation will also be discussed in the context of behaviors and gene regulation. Lastly, a major challenge in the field is the development of non-invasive biomarkers that can index brain iron status in newborn infants. Our recent discoveries in brain-derived exosomes from cord blood will be discussed in the context of biomarkers for brain iron status and neuroinflammation.

“Maternal Hyperinsulinemia: Shaping the Future Metabolic Health of Offspring”

Emilyn Alejandro
University of Minnesota, United States

Obesity and prediabetes are increasing concerns among women of reproductive age. While the negative effects of maternal hyperglycemia and obesity are known, the specific impacts of maternal hyperinsulinemia, associated with obesity and prediabetes, are unclear. To address this, we developed murine models with increased beta-cell mass to study the effects of maternal hyperinsulinemia independent of hyperglycemia or obesity. We also created a placental murine model lacking the insulin receptor to examine the lasting effects of placental insulin receptor loss on offspring metabolic health.

Our findings revealed that pancreatic beta-cell TSC2KO (enhanced mTOR signaling) mouse dams during pregnancy exhibited elevated insulin levels while maintaining normal blood sugar levels. A second model, beta-cell AKT, was also utilized to study the effects of maternal hyperinsulinemia. Offspring exposed to maternal hyperinsulinemia (HIP) from beta-cell TSC2KO dams displayed normal body weight when fed a regular diet (NCD) compared to control offspring. However, male HIP offspring demonstrated glucose intolerance at approximately 8 weeks of age, despite maintaining normal insulin sensitivity and secretion. The offspring exposed to maternal hyperinsulinemia (HIP) from beta-cell AKT dams exhibited increased body weight and glucose intolerance by 6 weeks of age, confirming that maternal hyperinsulinemia is sufficient to alter the metabolic health of the offspring.

Next, we tested the impact of placental insulin in the metabolic health trajectory of the offspring. Adult InsRKO ^{Placenta} mice maintained normal glucose homeostasis when fed a normal chow diet (NCD). However, under a high-fat diet (HFD) challenge, adult male InsRKO^{Placenta} offspring demonstrated lower body weight and mildly improved glucose homeostasis compared to controls. Notably, the improved glucose homeostasis in male InsRKO^{Placenta} offspring was associated with parity, suggesting a potential protective effect in the offspring of multiparous dams (hyperinsulinemic dams) under conditions of a diet-induced obesogenic challenge.

Overall, our study suggests that maternal hyperinsulinemia is sufficient to influence offspring glucose regulation. Additionally, placenta-specific insulin receptor deletion does not appear to have adverse effects on offspring glucose homeostasis in adulthood. Instead, there may be a mild and transient protective effect in the offspring of multiparous dams under conditions of a diet-induced obesogenic challenge. These findings contribute to our understanding of the complex interplay between maternal metabolic factors and offspring health, highlighting the importance of addressing maternal hyperinsulinemia in the context of reproductive health and child development.

Symposia

Symposium 1: Ion Channels in Reproduction and Pregnancy

Chairs: Ingrid Carvacho and Marcelo González-Ortiz

"The role of ion channels and receptors in the detection of early embryonic signals."

Joris Vriens
Katholieke Universiteit Leuven, Belgium

Background. Early embryo implantation is a complex phenomenon characterized by the presence of an implantation-competent blastocyst and a receptive endometrium. Embryo development and endometrial receptivity must be synchronized and an adequate two-way dialogue between them is necessary for maternal recognition and implantation. Proteases have been described as blastocyst-secreted proteins involved in the hatching process and early implantation events. These enzymes stimulate intracellular calcium signaling pathways in endometrial epithelial cells. However, the exact molecular players underlying protease-induced calcium signaling, the subsequent downstream signaling pathways and the biological impact of its activation remain elusive.

Methods. To identify gene expression of the receptors and ion channels of interest in human and mouse endometrial epithelial cells (EEC), RNA sequencing, RT-qPCR and in situ hybridization experiments were conducted. In Vitro and ex vivo calcium microfuorimetric experiments were performed to study their functional expression. A conditional PAR2 was used to determine the role of PAR2 in reproduction.

Results. Our results showed that trypsin evoked intracellular calcium oscillations in EEC of mouse and human and identified the protease-activated receptor 2 (PAR2) as the molecular entity initiating protease-induced calcium responses in EEC. In addition, this study unraveled the molecular players involved in the downstream signaling of PAR2 by showing that depletion and re-filling of intracellular calcium stores occurs via PLC, IP3R and the STIM1/Orai1 complex (1). The progesterone specific PAR2 cKO mice showed an increased number of miscarriages and significant prolonged time period to the first litter. Finally, in vitro experiments in the presence of a specific PAR2 agonist evoked an upregulation of the 'Window of implantation' markers in human EEC.

Conclusions. These findings provide new insights into the blastocyst-derived protease signaling and allocate a key role for PAR2 as endometrial sensor for signals released by the developing blastocyst.

Keywords. Early embryo implantation, protease activated receptor 2, serine proteases

Funding. Research Foundation-Flanders FWO, (G.0D1417N, G.084515N, G.0A6719N) and the Research Council of the KU Leuven (C14/18/106)

“The spatial distribution of CFTR-rich ionocytes cells in the postnatal mouse airway development”

Sandra Villanueva
Universidad San Sebastián, Chile

Ionocytes are a new type of rare airway epithelial cell expressing Ascl3 and Foxi1 transcription factors, as well as near 50% of Cftr-transcripts. Despite, the intriguing high expression of the CFTR chloride channel, the protein mutated and causing cystic fibrosis, the most common human genetic disease, the role and location of ionocytes remain unexplored. We aimed to describe ionocyte ontogeny and distribution within the mouse airways to understand their function in airway homeostasis.

Mice (C57Bl6/J) were bred and maintained in the Specific Pathogen Free mouse facility of CECs with access to food and water ad libitum. We used 1,14,21 and 28-days-old wild-type mice, and 6 and 8-week-old wild-type and CftrΔF508/ΔF508 animals, the last corresponding to a model of cystic fibrosis. Tracheas were sliced in frontal sections, and ionocytes were identified by immunofluorescence against FOXI1. Animal procedures were approved by the institutional IACUC (CECS-2022-03).

We found that FOXI1+cells had a triangular shape with a basolateral process, appeared postnatally close to the submucosal glands (SMG) and their quantity increased drastically between 6 and 8 weeks of age. The amount of FOXI1+cells cells in adult tracheas decreased towards the distal section. FOXI1+cells were often observed in the epithelia around the collecting duct exit and in the collecting duct epithelium of the SMG. No differences between WT and CftrΔF508/ΔF508 were observed.

In conclusion, our study provides new insights into the localization and distribution of ionocytes. These cells might play a role regulating mucus composition in upper airways. We suggest that age-dependent changes in cell quantity might reflect the need of increased CFTR function in adult stages, and that quantity and localization of ionocytes are not depending on functional CFTR channels. Further research is needed to fully understand the function of ionocytes in the airways and their role in respiratory diseases such as cystic fibrosis

Keywords: ionocytes, airways, postnatal- development.

Funding: Proyecto Postdoctoral 3220672 Proyecto FONDECYT 1221257.

“Dissecting the contribution of ion channels in the oocyte-to-embryo transition in mammals.”

Ingrid Carvacho
Universidad Católica del Maule, Chile

Background: Oocyte maturation or the acquisition of meiotic competence requires a controlled expression of proteins that supports this process in preparation for fertilization. Both, oocyte maturation and fertilization are determined by a highly regulated ion homeostasis. Several ion channels, regulating diverse cellular processes, have been reported to be expressed in oocytes and eggs from different species, including mammals. Fertilization starts with the release of the sperm-specific phospholipase ζ (PLCζ) in the mature oocyte, egg. Ca^{2+} influx is required to accumulate Ca^{2+} in the oocyte in preparation for fertilization, as well as to refill its intracellular stores during fertilization, supporting Ca^{2+} oscillations and egg activation. The egg activation includes the formation of the pronucleus, cortical granule exocytosis, polyspermy blockade, completion of meiosis II, and the transition to embryonic development. Ion channels are responsible for Ca^{2+}, Mg^{2+}, H^+ and other ions gradients in immature oocytes and eggs, underlying oocyte-to-embryo transition in mammals.

Methods: Using molecular biology, animal models, confocal microscopy, and electrophysiological tools, we tested the expression and function of ion channels in mouse oocytes and eggs, and analyzed their role in oocyte maturation and fertilization.

Results: We found that the non-selective cation channels TRPV3 and TRPM7, and the voltage-gated Ca^{2+} channel Cav3.2 are expressed in the plasma membrane of oocytes and eggs. The expression of these channels varies during the acquisition of meiotic competence (oocyte maturation). We also found that the H^+ channel Hv1 is expressed in oocytes and eggs. The expression of a diversity of ion channels suggests unexplored and complex mechanisms underlying ion homeostasis in oocytes and eggs.

Conclusions: Our data set up starting points to discover new proteins and/or protein complexes participating in ions-mediated signaling and mammalian reproduction.

Key words: Oocyte maturation, fertilization, ion channels

Funding: NIH RO1 HD092499; FONDECYT 1221308

Acknowledgments: FONDEQUIP ANID EQM200122

“Role of calcium-activated potassium channels in gestational diabetes.”

Marcelo González-Ortiz
Universidad de Concepción, Chile

Introduction: The calcium-activated potassium channels (KCa) have a relevant role in the regulation of vascular tone, especially related to endothelium-derived relaxation and vasodilation. In the human placenta has been described the expression of large conductance (BKCa) and intermediated conductance (IKCa) potassium channels, which expression decreases in preeclampsia. In gestational diabetes mellitus (GDM) there is placental endothelial dysfunction and oxidative stress, but still is not reported the potential changes in KCa expression or function. Aim: To determine the plasma membrane potential and BKCa expression changes in fetal endothelium from GDM pregnancies.

Methods: From normal and GDM pregnancies (informed consent signed) were obtained the human umbilical vein endothelial cells (HUVECs), were grown in a primary culture medium until passage 2 previous to the determination of plasma membrane potential and BKCa expression. The changes in plasma membrane potential were determined using the fluorescent dye DiBAC(4)3, quantified in live cells maintained in culture conditions (37ºC, 5%CO2)(Incucyte system). The BKCa expression (α and β1 subunits) was determined by immunofluorescence and confocal microscopy.

Results: In HUVECs from GDM there is a significant ($p<0.05$) 34% decrease in DiBAC(4)3 fluorescence, compared with normal cells. Lower DiBAC(4)3 fluorescence indicates a significant hyperpolarization, which is associated with higher expression of α and β1 subunits of BKCa. The incubation of cells with iberiotoxin (BKCa inhibitor) increased the hyperpolarization in normal, but induced depolarization in GDM HUVECs. The incubation with insulin (0.1-100 nM) induced hyperpolarization at 10 and 100 nM in normal HUVECs, and a significant depolarization at 1nM in GDM cells. The depolarization in GDM is associated with overexpression of BKCa α compared with β1 subunits.

Discussion: In GDM HUVECs the high expression of BKCa α subunit is associated with basal hyperpolarization and iberiotoxin-induced depolarization. The alterations of insulin's effects in GDM could be associated with plasma membrane dysfunction.

Fundings: VRID-Multidisciplinario 2020000157MUL (U. de Concepción).

Keywords: Gestational diabetes, placenta, potassium channels, insulin, hyperpolarization

Symposium 2: Fetal Cardiovascular Programming: Mechanisms and Interventions

Chair: Emilio Herrera

"Lactation as period of intervention to reduce cardiovascular programming induced by undernutrition."

Dra. Silvia Arribas
Universidad Autónoma de Madrid, España

Background. Low birth weight (LBW) caused by maternal undernutrition, obstetric complications or prematurity, associates with increased risk of cardiometabolic diseases (cardiometabolic programming), being oxidative stress a key mechanism. Breastfeeding is a well-known protective factor reducing cardiometabolic risk, ensuring a physiological growth and relevant bioactive factors. We aimed to explore the role of lactation in cardiometabolic programming. Methods. We used a rat model of LBW induced by maternal undernutrition during gestation (MUN), where male offspring develops hypertension in adult life, and at weaning show a generalized antioxidant deficiency (1) and cardiovascular hypertrophy (2) (Ethical approval PROEX 04/19). At birth MUN and control pups were exchanged and nursed by a foster mother during lactation, evaluating at weaning changes in growth & adiposity, cardiovascular structure (echocardiography, microscopy) and cardiac gene expression (RT-PCR). We also analyzed if MUN dam supplementation during lactation with an extract from cocoa shell (CSE, enriched in antioxidants) prevented offspring hypertension and vascular remodeling (pressure myography). Results. During exclusive lactation period MUN males, but not females, accelerated growth in association with cardiovascular and adipocyte hypertrophy and up-regulation of cardiac angiotensinogen gene expression. Fostering MUN males on Control dams significantly reduced weight gain rate, cardiovascular and fat hypertrophy and reversed cardiac angiotensinogen gene up-regulation, while nursing Control pups by a MUN dam, had no effect. CSE-supplementation of MUN dams during lactation prevented the development resistance artery remodeling and hypertension in adult male offspring. Conclusions. 1) In the process of cardiometabolic programming, both fetal and lactation periods are important for the development of altered phenotype. 2) Maternal supplementation during lactation can counteract alterations induced by a fetal insult. Thus, lactation period is a key intervention window to counteract fetal programming.

Keywords: fetal programming, lactation, antioxidants, catch-up growth, cardiometabolic diseases, undernutrition, Cocoa shell

Financing: Ministerio de Ciencia, Innovación y Universidades (Spain), grant number RTI2018-097504-B-I00, cofinanced with FEDER funds

Acknowledgments: FOSCH Research Group

“Perinatal hypobaric hypoxia and postnatal pulmonary vascular adaptations.”

Roberto Reyes
Universidad de Chile, Chile

Pulmonary arteries have intrinsic responsivity to hypoxia, mainly contraction and hyperplasic thickening of vascular wall, detectable early in the fetal life and persisting during the postnatal life. Hypobaric hypoxia during gestation and early postnatal life may program and modify the vasoconstrictor and mitogenic response of pulmonary circulation to new hypoxic challenges and other stimuli. Changes in NO- and Ca2+- signaling contribute to these changes. Nevertheless, in species with evolutive ancestry under hypobaric hipoxia, this maladaptive programming in pulmonary circulation seems attenuated and protect them against pulmonary hypertension (PHT).

Newborn sheep gestated and born under high-altitude hypoxia develop PHT with enhanced hypoxic pulmonary vasoconstriction (HPV) whilst high-altitude newborn llama do not increase their basal pulmonary arterial pressure, and their HPV is decreased. Both highland sheep and llama have increased pulmonary arterial wall thickness, but at equivalent altitudes the degree of medial layer growth is lower in the llama than the sheep. NO signaling is unable to prevent the increase of pulmonary arterial pressure under basal and acute hypoxic conditions in the newborn sheep, whilst it contributes to prevent the increase of these variables in the llama. Moreover, the efficacy of the nitric oxide signaling system in the llama is improved on steps downstream to NO synthesis, like Ca2+-sensitization, whilst it is blunted in the sheep. Another mechanism potentially modified by hipoxia in utero is Ca2+ handling. Both high-altitude sheep and llama have up-regulated Orai1 Ca2+ channels expression, but its function is enhanced in the sheep whilst it is silenced in the llama.

Evolution under hypobaric hipoxia probably lead to genetic adaptations allowing defense against prevent maladaptive programming of the neonatal pulmonary circulation in rersponse to perinatal hipoxia: these adaptations are related to mechanisms that regulate both the sensitivity to Ca2+ determined by NO signaling and maybe Ca2+ entry though Orai1 channels

Keywords: Perinatal Hypoxia, Pulmonary circulation, Nitric oxide signaling, Ca2+

Financing: FONDECYT 1210504 and Puente ICBM.

Acknowledgments: We are grateful of technical assistance of Mr Carlos Brito and Gabino Llusco all along these years

“Cerebrovascular dysfunction by intrauterine hypoxia: early origins on Blood-Brain Barrier permeability.”

Alejandro González-Candia
Universidad de O´Higgins, Chile

Background. The blood-brain barrier (BBB) is centrally positioned within the neurovascular unit (NVU) and is formed by endothelial and mural cells. BBB breakdown leads to leakages of components into the CNS, contributing to neurological deficits. Although studies have described the effects of gestational hypoxia in fetal development, there is no data regarding the impact of gestational hypoxia on BBB permeability. We have hypothesized that gestational hypoxia modifies the permeability of the BBB. Furthermore, we will identify the molecules of junctional complexes of the BBB that are affected by gestational hypoxia.

Methodology. All animal experimentation was approved by the Institutional Animal Care Committee (certificate 20418-MED-UCH). Five newborn Guinea Pigs were assigned to normoxia (Nx), and Hypoxia groups (Hx). At gestational day (GD) 30, both groups were introduced to a hypobaric chamber in conditions of normoxia (Nx, 720 torr) or hypoxia (Hx, 470 torr) until delivery, G70 animals were euthanized, and fetal brain was collected. The gene and protein expression related to the integrity was evaluated by qPCR, western blot, and immunohistochemistry. The permeability is quantified by immunolocalizing albumin in brain tissue; a non-parametric student t-test was used. Significative differences were considered when $p<0.05$.

Results. Quantifying the albumin-immunopositive areas showed a significant increase in the brain parenchyma in Hx compared with Nx vessels. In addition, the expression of genes and proteins related to the neuro-endothelial permeability showed a decrease in the expression of claudin 5, 12, and ZO-3 when we compared the Nx group with the Hx group; however, the expression of ZO-2 was significantly lower in the Hx group compared to the Nx group.

Conclusions. Hypoxia during gestation dramatically impacts fetal brain permeability. However, advances in understanding how gestational hypoxia induces variations in the expression of genes and proteins involved in the integrity of the cerebrovascular network remain widely unexplored.

Keywords: Paracellular Permeability, Tight Junctions, Fetal growth restriction

Financing: Fondecyt de Inicio 11200798 and Fondecyt Regular 1201283

Acknowledgments: We thank Mr Marcelo Barrales and Tamara Jimenez for their excellent technical assistance.

“Molecular interventions for preventing the fetal hypoxia-induced cardiovascular programming.”

Bernardo Krause
Universidad de O´Higgins, Chile

In the clinic, fetal growth restriction (FGR) is defined by a fetal weight below the 10th percentile. However, new evidence shows that impaired intrauterine growth may affect several neonates born > 10th percentile, especially late in pregnancy, which may be missed from perinatal surveys for preventing adverse outcomes. FGR remains a leading cause of perinatal morbidity and mortality, affecting ~10% of pregnancies, but ranging from 5 to 25% depending on the population surveyed, with a higher prevalence among pregnant women of low socioeconomic status. These statistics are important because FGR programs a higher cardiovascular risk later in life, an effect that may be mediated by accelerated aging at molecular, structural, and functional levels.

Several studies show that vascular dysfunction is found at birth in chorionic and umbilical arteries from FGR pregnancies. Fetal hypoxia, oxidative stress, and altered blood flow patterns (e.g. shear stress) are key clinical markers associated with FGR. However, how these factors interact and impose an epigenetic program on endothelial function in FGR offspring remains elusive.

Combining studies in isolated organs with research at the molecular, cellular, and epigenetic levels, this presentation will highlight our discoveries on markers of endothelial dysfunction in humans, guinea pigs, and chicken embryo vessels. The induction of ancillary vasodilator pathways in response to glutathione precursors and the use of epigenetic-based (i.e. miRNA) prenatal treatments will also be discussed. Combined, our data support that human umbilical artery endothelial cells can be used as a surrogate measure to explore a fetal origin of cardiovascular programming. Further, there is a clear role for epigenetic mechanisms activated in FGR providing the opportunity for molecular interventions against fetal hypoxia-induced origins of future cardiovascular health risks.

Keywords: epigenetics, fetal growth, vascular, cardiovascular, hypoixa

Financing: Acknowledgment to Fondecyt 1220421, Proyecto Multidisciplinario MDM2021004, and The British Heart Foundation RG/17/8/32924.

Symposium 3: The Key Role of the Placenta in Developmental Programming

Chairs: Gloria Barbosa Sabanero and María Luisa Lazo de la Vega Monroy

"Placental lipidomic analysis of newborns with idiopathic alterations in birth weight from mothers with and without obesity."

Gloria Barbosa Sabanero
Universidad de Guanajuato, México

Background. Birth weight is a surrogate marker of risk for the development of metabolic diseases in adulthood. Alterations in birth weight have been related to maternal obesity (1). The placenta study allows to evaluate prenatal exposures in DOHaD context (2). Underlying mechanisms and placental molecular disturbances of idiopathic alterations in birth weight or associated with maternal obesity are not known. Novel tools such as lipidomics, could help to a better understanding of placental physiology (3) even in obesity conditions. Our aim was to evaluate the lipidomic profile of placenta of newborns with idiopathic alterations in birth weight and maternal obesity.

Methods. Placental samples (n=90) from women (18-35 years old) with pregestational obesity or normal weight were included and classified according to the weight of the newborn in small (SGA), adequate (AGA) and large for gestational age (LGA), and LGA-OB, from mothers with obesity. Lipid extraction followed by non-targeted lipidomic analysis by high performance liquid chromatography coupled to mass spectrometry (UPLC-QTOF MS) was performed. Lipid comparison was performed by ANOVA. Approved by Institutional ethics committee (CIBIUG-P71-2021).

Results A total of 185 placental lipids were pre-identified, corresponding to ten different families. When comparing the groups of idiopathic alterations in birth weight from normal weight mothers, we observed that the lipids with highest discriminating power were some ceramides, triglycerides, sphingomyelin, diacylglycerols, phosphatidylethanolamines, DHA, and phosphatidylcholines. Being the lipidome of LGA very different from lipidome of AGA and SGA groups. Six altered metabolic pathways were found, being the pathway of glycerophospholipid metabolism with the greatest impact. The comparison between LGA-OB vs LGA showed significant differences in 70 lipids, observing an increase in triglycerides and diacylglycerols in LGA-OB group.

Conclusions. The lipid profile in the placenta is influenced by maternal obesity, which suggests an impact on the offspring that could be a metabolic programming mechanism.

Keywords: Placenta, Lipidomics, Obesity, Birthweight alterations

Financing: Support by DAIP-UG (CIIC 223/2022), IDEAGTO/CONV704672022UG and ECOS-NORD (FORDECYT-PRONACES 195/2021/315669).

“Metabolomic analysis of placenta form newborns with idiopathic birthweight alterations with and without maternal obesity.”

María Luisa Lazo de la Vega Monroy
Universidad de Guanajuato, México

Background. Adverse intrauterine factors induce changes in the fetus, predisposing it to develop chronic-degenerative diseases in adulthood. Neonates born small (SGA) or large (LGA) for gestational age have increased risk of metabolic and neuropsychological diseases in adulthood. The placental physiology and metabolism links maternal alterations with this fetal programming. Therefore, a metabolomic approach can help to understand the placental response to maternal conditions, identifying potential biomarkers for the early prognosis of programmed diseases in utero. Our aim was to perform a metabolomic analysis of placenta from newborns with idiopathic alterations in birthweight with and without maternal obesity.

Methods. Placental samples from 18–35-year-old women with normal weight with small (SGA, n=21), adequate (AGA n=45), and large for gestational age (LGA n=15) newborns, and LGA from women with pregestational obesity (LGA-OB n=9) were included. Metabolomic analysis was performed by UPLC-QTOF MS. ANOVA, PCA, and PLS-DA analysis was done. Study approved by Institutional ethics committee (CIBIUG-P71-2021).

Results A total of 172 metabolites were pre-identified, mostly lipids, such as sphingomyelin, diacylglycerols, phosphatidylserines, phosphatidylethanolamines, ceramides, phosphatidylinositol, lysophosphatidylethanolamines, monoglycerides, and fatty acids. Tripeptides, dipeptides, amino acids, nucleotides, carnitines, oxidative stress markers (glutathione), prostaglandins, leukotrienes, and vitamin D precursors were also pre-identified. No placental metabolome separation among the groups (AGA, SGA, LGA, and LGA-OB) was found. When comparing metabolites in placentas without maternal obesity (SGA, AGA and LGA), pyroglutamic acid, nitrotryptophan, and glycerophosphatidylcholine P-42: 4 (PE(P-42:4)) were higher in the LGA group. As well, Pyroglutamic acid, oxidized glutathione, Beta-citril-L-Glutamate, cytidine, and succinyl adenosine, were higher in LGA-OB, while nitrotritophan was higher in LGA compared to AGA.

Conclusions. Placental metabolites are different in LGA and LGA-OB compared to AGA newborns. Interestingly, most of the identified metabolites are lipids. These results may shed light into the potential placental mechanisms of birthweight establishment, particularly for elevated birth weight.

Keywords: placenta, metabolomics, birthweight

Financing: This work was supported by DAIP-UG (CIIC 131/2023), IDEAGTO/CONV704672022UG and ECOS-NORD (FORDECYT-PRONACES 195/2021/315669).

“Alterations in placental steroid synthesis in women with obesity.”

Manuel Maliqueo
Universidad de Chile, Chile

Gestational obesity induces several adaptations to maternal physiology that have consequences for fetal development, increasing the risk of developing obesity and its complications, such as non-alcoholic fatty liver disease, hypertension, and diabetes, suggesting that they are established as early as fetal life. Placenta, as an endocrine organ, produces steroid hormones that regulate maternal metabolism, nutrient transport, and fetal growth. Therefore, alterations in placental steroid synthesis affect normal fetal development with long-term consequences. In this regard, we have focused on searching and understanding maternal and fetal mediators that account for placental adaptations in women with gestational obesity. Women with gestational obesity exhibit elevated levels of androgens, dehydroepiandrosterone-sulfate, testosterone, and lower levels of progesterone and estrone. Androgen levels are influenced by maternal insulin resistance, male fetal sex, and levels of TNF-alpha in umbilical cord blood. Interestingly, the expression of enzymes involved in progesterone and estrogen synthesis, such as CYP11a and CYP19, show a dimorphic pattern in the placenta of obese pregnant women (Ethical approval "CEISH, Facultad de Medicina, Universidad de Chile 033-2013"). Transcriptome analysis in mice models shows alterations in estrogen response in the placenta from animals with gestational obesity, whereas maternal hyperandrogenism affects the expression of genes related to placental development (Ethical approval "CBA, Facultad de Medicina, Universidad de Chile 1040"). On the other hand, in vitro studies using a model of placental explant stimulated with testosterone show that the protein expression of fatty acid transporter (FATCD36) is increased in the placenta of female fetuses and decreased in the placenta of male fetuses. Therefore, maternal obesity alters maternal levels of sex steroids, which seem to regulate placental development and its function resulting in mechanisms that could affect fetal development with long-term consequences for the offspring.

Keywords: placenta, steroid, obesity, androgen

Financing: FONDECYT 11130250, 1181798 and ENL 031/2017

“Effect of psychotropic drugs on placental transporters”

Martha Sosa Macías
Instituto Politécnico Nacional-CIIDIR Durango, México

Background. Fluoxetine and sertraline are the most frequently used antidepressant medications during pregnancy1. These drugs may be associated with an increased risk of birth defects mainly congenital heart defects2. The impact of chronic administration of these drugs on placental transporters for hormones and nutrients is poorly understood.

The aim of this work was to assess the effect of fluoxetine and sertraline exposure on the messenger RNA (mRNA) expression of placental transporters in pregnant rats Wistar.

Methods. The work was approved by an ethics committe. Studies were conducted on gestational days (GD) 11, 16 and 20, following a treatment with fluoxetine (2.06mg/kg) or sertraline (10mg/kg), or the vehicle every 24h after weaning. The doses were administered orally with an esophageal cannula. The placental expression of transporters Lat1, xCT, RFC, Oatp4a1, Folr1 was evaluated through qRT-PCR. The Kruskal-Wallis and the Mann-Whitney U tests were used to determine the statistical differences ($p<0.05$) in gene expression, using the $\bar{x}$ RQ value.

Results. At GD 11 and 16, fluoxetine induces the expression of Oatp4a1 and Lat1, respectively and represses them at GD 20 ($p<0.05$). RFC expression was increased by fluoxetine at all GD ($p<0.05$) while xCT was expressed in similar levels in control and exposed groups. At GD 11 the expression of Lat1 was higher in the group treated with sertraline than the control group ($p<0.05$), and the Folr1 expression was increased by sertraline at all GD ($p<0.05$).

Conclusions. The present study suggested that fluoxetine and sertraline alter the expression of placental transporters. In particular, RFC and Folr1 were induced throughout gestation, which are involved in the regulation of intracellular folate concentrations. Further studies must be performed to define the impact of these psychotropic drugs on placental physiology and, therefore, fetal development, both in the model studied and in humans.

Keywords: Placenta, Transporters, psychotropic drugs

Financing: This research was funded by grants from the Secretaría de Investigación y Posgrado (SIP) of Instituto Politécnico Nacional (SIP 20231237 and SIP 20230626), México.

“Evaluation of maternal and paternal nucleotide variations in preeclampsia development.”

Carlos Galaviz Hernández
Instituto Politécnico Nacional-CIIDIR Durango, México

Background. Preeclampsia is a pregnancy disease with high rates of maternal-fetal mortality and morbidity. Chymase (Chy) is responsible of the production of angiotensin II (ANG II) and endothelin in placenta. Chy is observed in the endothelium of preeclamptic patients. The father is important in preeclampsia development. This work evaluated the potential association of maternal, paternal or combined (maternal+paternal) genetic variations in Chy encoding gene CMA1 with preeclampsia.

Methods. This case-control study was approved by the ethics and research committees of the Hospital de la Mujer in Durango, México and included patients with preeclampsia and healthy pregnant women (HPW) with their respective partners. Genotyping of the promoter SNV rs1800875 (-1903 G/A) and the 3´UTR repeat (TG)n(GA)m in mothers and fathers was performed. CMA1 gene expression was evaluated in placentas. Serum Chy and IgE were measured. Descriptive statistic was done. Multivariate regression analysis evaluated association. Correlations were analyzed with Spearman’s test.

Results. Eighty patients with preeclampsia and 61 HPW with their respective partners were enrolled. No association between SNV rs1800875 with preeclampsia was observed. The analysis of 3´UTR repeat (TG)n(GA)m, showed significant differences in the frequency of fragments 34 and 35 between cases and controls in the women and combined groups. No differences in CMA1 gene expression were observed. Serum Chy was higher in cases ($p=0.048$), serum IgE were higher in controls ($p=0.027$). The expression of CMA1 gene and personal history of preeclampsia were correlated ($r2=0.4227$, $p=0.012$). The serum Chy correlated with the CMA1 gene expression ($r2=0.579$, $p=0.008$). The SNV rs1800875 in the control group was correlated with the serum IgE ($r2=0.478$, $p<0.05$).

Conclusions. No associations were observed between SNV rs1800875 with preeclampsia. Significant differences in the frequency of fragments 34 and 35 were identified between cases and controls in the mothers and combined groups. No paternal influence in preeclampsia was demonstrated.

Keywords: Preeclampsia, paternal, chymase.

Financing: Secretaría de Investigación y Posgrado (SIP), Instituto Politécnico Nacional, México. SIP 20230626 y SIP20231237.

Symposium 4: Biomarkers in Reproductive Biology

Chair: Dolores Busso

"Using Biomarkers in Oral Fluids as a Promising Strategy to Predict Diseases of Pregnancy."

Alejandra Chaparro
Universidad de los Andes, Chile.

Early and innovative diagnostic strategies, with a minimally invasive approach, are required to predict the future risk of developing pre-eclampsia (PE) and gestational diabetes mellitus (GDM) in pregnancy. This research aims to evaluate the diagnostic performance of oral biomarkers (extracellular vesicles, non-coding RNAs, inflammatory and placental mediators) to predict early pregnancy women's risk for developing PE or GDM. In addition, to establish the periodontal disease status and their association with GDM and PE. A prospectively collected, retrospectively stratified cohort study was conducted, with pregnant women recruited at 11–14 weeks of gestation. Physical, obstetrical, and oral health data were recorded. Gingival crevicular fluid and saliva were collected for the biomarker screening and validation. Extracellular vesicles, non-coding RNAs, inflammatory and placental mediators were identified in oral fluids by RNA sequencing, proteomics, LUMINEX and RT-qPCR approaches. Logistic regression classification models were developed, and the performance diagnostic accuracy of the models was evaluated. As a result, the predictive models using oral fluids during the first trimester of pregnancy are highly accurate in discriminating the future risk of GDM and PE. Moreover, the severity of periodontal disease was associated with GDM diagnosis. Thus, extracellular vesicles, placental and inflammatory biomarkers detected in oral fluids may be helpful as an aid in identifying pre-symptomatic women who subsequently develop PE or GDM. the present results provide insights into the potential capacity of first-trimester oral extracellular vesicles or their proteomics or transcriptomic cargo as early predictors of gestational diabetes mellitus and preeclampsia in clinically healthy pre-symptomatic pregnancies.

Keywords: Extracellular vesicles, gestational diabetes, preeclampsia, periodontitis, oral fluids, diagnostics, biomarkers

Financing: Fondecyt Regular ID1211471, CORFO 22CVID-206879.

Acknowledgments: ANID, Fondecyt Regular ID1211471, CORFO 22CVID-206879.

"Small Extracellular Vesicles from Menstrual Fluid: a New Biological Source for Non-invasive Biomarkers of Endometriosis?"

Lara Monteiro
Universidad de los Andes, Chile.

Introduction: Endometriosis is a chronic, debilitating gynecological disorder characterized by the presence of endometrial cells outside of their normal uterine location. It affects approximately 10% of reproductive-age women worldwide, and it is associated with pelvic pain and infertility in 30-50% of the affected women. Endometriosis remains an enigmatic disease of unknown etiology, with delayed diagnosis due to the inexistence of an accurate early-diagnostic method.

Materials and Methods: Small extracellular vesicles (sEV) isolated from peripheric blood and menstrual blood from the same patients were compared; sEV isolated from menstrual blood (MB) obtained from healthy patients and (n=17) and endometriosis (n=7) patients were compared. sEV were isolated by ultracentrifugation and particles' size and concentration were determined by Nanoparticle Tracking Analysis. Populations of CD63, CD81 and CD9 surface markers were determined by flow cytometry and sEV morphology was analysed by transmission electron microscopy (TEM). This study was reviewed and approved by the Ethical Scientific Committee of Universidad de los Andes (CEC2022107). Confidential informed consent was signed by all patients.

Results: MB had significantly higher concentration of sEV compared with PB. No significant changes were observed regarding the concentration of sEVs comparing controls and endometriosis women ($5.9x10^9$ and $6.9X10^9$, respectively; p=0.98). A mode particle diameter of 170 nm and 182 nm was obtained for controls and endometriosis, respectively (p=0.43). Moreover, we identified the presence of cup-shaped particles, characteristic of small extracellular vesicles for both groups and by using flow cytometry we have further demonstrated that the obtained sEV are positive for CD9, CD63 and CD81 protein surface markers. A significant decrease in the CD63 surface marker was observed in the endometriosis group (p=0.02).

Conclusions: These results validate MB-derived sEV as a potential new source for identifying biomarkers for a non-invasive diagnosis endometriosis.

Keywords: endometriosis, menstrual blood, small extracellular vesicles, non-invasive diagnosis

Financing: ANID CHILE FONDECYT REGULAR #1230932; ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024; Universidad de los Andes FAIN #2022001

"Prenatal Stress and Neurodevelopment: Can Small Extracellular Vesicles Serve as Mediators and Biomarkers?"

Federico Batiz
Universidad de los Andes, Chile.

Maternal psychological distress during pregnancy, also known as prenatal stress (PS) increases the risk of poor neurodevelopmental outcomes in the offspring. The mechanisms and mediators by which maternal stress is communicated to the fetus remain poorly understood. In addition, there are no early biomarkers to predict neurodevelopmental outcomes in the context of PS. Our group is investigating a novel mother-to-fetus (and placenta) communication pathway mediated by small extracellular vesicles (sEVs) that might regulate fetal neurodevelopment under PS conditions. sEVs are a heterogeneous population of membrane-bound vesicles of varying biogenesis, size, content, and bioactivity. sEVS are specifically packaged with a complex cargo of molecules of different types, comprising lipids, proteins, and RNAs; they are actively released into biofluid compartments, such as the bloodstream, and can regulate the physiology of distal target cells. Thus, sEVs represents a complex integral signaling pathway mediating intercellular communication. Moreover, most of the cargo of EVs is composed by products from the donor cell and is cell-type and cell-status specific. Thus, the content of EVs is considered a "fingerprint" of the donor cell that reflects its physiological or pathophysiological status and, as such, sEVs have been proposed as useful biomarkers for different conditions and pathologies. We have developed a rat model of PS by repetitive movement restraint, and we are studying the consequences on fetal neurogenic process. In addition, we are investigating whether maternal sEVS can mediate these changes. These studies are being complemented by in vitro assays using primary cultures of neural stem/progenitor cells. On the other hand, we have studied the consequences of the exposure to a high magnitude earthquake and the COVID-19 pandemic on maternal psychological distress (depressive symptoms and perceived stress) during pregnancy and changes in neurodevelopmental outcomes in the offspring at different postnatal stages. Our results strongly suggest that sEVs can mediate stress-related signals and have the potential to be used as biomarkers of PS-induced neurodevelopmental changes.

Acknowledgement: ANID-Fondecyt 1211384 Grant (LFB); ANID-COVID0706 Grant (LFB); ANID-FONDEF ID19I10116 (UW); ANID-Basal Funding for Scientific and Technological Center of Excellence #FB210024 (GN, LJM, UW, SEI, LFB); FAI-UANDES, FAMED-UANDES & IMPACT PhD Scholarships (SO, MMR)

"Circulating Small RNAs as Potential Biomarkers of Fetal Programming-related Processes."

Erika Chavira Suárez
Universidad Nacional Autónoma de México.

Background. In conjunction with other molecules and non-coding RNAs (long and circular RNAs), the small non-coding RNAs (sncRNAs) regulate gene expression in tissues and organs such as in the placenta. MicroRNAs (miRNAs) are the most abundant type of sncRNAs in the maternal circulation, and some of them may represent pregnancy-related compartments and processes. However, it remains unclear whether the expression of sncRNAs in peripheral blood can be used as a tool to monitor pregnancy and diagnose perinatal complications that affect developmental programming.

Methods. Using small RNA sequencing, we performed a large-scale sncRNA profiling of maternal plasma during and after pregnancy (n=8 pregnant women over three pregnancy periods and one post-pregnancy period) and compared it with that of non-pregnant women (n=10). Fetal and placental anthropometry by ultrasound was correlated with changes in the most abundant type of sncRNAs in the maternal circulation. The INMEGEN's Review Board (register 07/2018/I) and the Ethics in Human Subjects and Research Committees of Mexico City Secretaria de Salud (register number: 210/010/31/17) approved the work.

Results. miRNAs were the most abundant type of sncRNAs (number of identified miRNAs= 723) in the maternal circulation, followed by tRNA-derived small RNAs (tsRNAs= 355), Y-RNAs (247), small nuclear RNAs and small nucleolar RNAs (snRNAs and snoRNAs= 88), and piwi-interacting RNAs (piRNAs= 19), showing specific temporal changes during normal pregnancy. Interestingly, we identified tsRNAs potentially derived from mitochondrial tRNAs and tRNAVal,Gly,Glu,Pro,Lys. Consistent with our previous work, the global expression of circulating miRNAs in pregnant women was under-expressed in pregnant women compared to non-pregnant women at different gestational ages. Finally, specific circulating miRNA signatures of fetal and placental growth were found.

Conclusions. Our results show evidence of sncRNAs in the maternal circulation that reflect pregnancy-related processes, which can be used in future studies to assess the developmental programming of the offspring.

Keywords: small non-coding RNAs, pregnancy, maternal circulation, sRNA-seq

Financing: CONACYT-SEP [grant A1-S-35245]; Fundación Gonzalo Río Arronte [grant S.633]; SECTEI [grant 253/2019] Mexico; INMEGEN [grant 07/2018/I]; FUNSALUD [grant PI1000d]; and CONACYT [grant 319605]

Acknowledgments: We thank the Sequencing Unit of the Instituto Nacional de Medicina Genómica, México, for technical assistance.

Symposium 5: The Janus Face of Polyphenols

Chair: Cristiane Matté

“Maternal intervention with resveratrol: the good, the bad, the ugly”

Carlos Alberto Ibáñez
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México

Background. Maternal obesity and protein restriction lead to oxidative stress, defined by increased levels of reactive oxidant species (ROS) and/or decreased antioxidant defenses. Maternal oxidative stress before and during pregnancy is a major determinant of the offspring’s predisposition to insulin resistance and obesity. Resveratrol is a natural polyphenol with ROS inhibitory/scavenging and anti-inflammatory properties. We aim to determine whether maternal supplementation with resveratrol counterbalances oxidative stress and prevents adverse metabolic outcomes in the offspring of mothers having poor nutritional conditions.

Methods. Through several experiments, obese and protein-restricted dams, have been supplemented with resveratrol at 20 mg/kg/d one month prior to and during pregnancy. Several physiological effects of maternal resveratrol supplementation have been evaluated in the mother, placenta, and male and female offspring at different stages of development, including gestation, birth, weaning, puberty and adulthood.

Results. Resveratrol supplementation in obese and protein-restricted mothers reduced maternal oxidative stress with some metabolic improvements, including functional and structural responses in the placenta. The Good: maternal resveratrol intervention reduced fetal oxidative stress and postnatal insulin resistance, dyslipidemia, and excessive adipose tissue accumulation induced by adverse maternal nutrition. The Bad: resveratrol supplementation led to sex-specific beneficial effects, suggesting different effectiveness of this intervention in male and female offspring. The Ugly: despite oxidative stress having deleterious effects on early development, moderate amounts of ROS are essential for specific cell functions, therefore, maternal excessive resveratrol doses might interfere with an offspring’s adequate development.

Conclusions. Maternal resveratrol supplementation before and during pregnancy reduces oxidative stress associated with adverse maternal nutrition and decreases offspring metabolic outcomes related to obesity. However, further experimental research is needed to understand the offspring’s sex-specific response to maternal resveratrol supplementation in light of an adequate balance between ROS levels and antioxidant defenses needed for an optimal gestational environment.

Keywords: Resveratrol Intervention, Maternal Obesity, Maternal Protein Restriction

Financing: Newton Fund RCUK-CONACyT (Research Councils UK—Consejo Nacional de Ciencia y Tecnología). I000/726/2016 FONCICYT/49/2016

“Parental consumption of Blackberry polyphenols or orange juice effects on breast cancer programming”

Thomas P. Ong
Universidade de São Paulo, Brazil

Breast cancer is a global public health problem. Accumulating epidemiological and experimental evidence show that this disease could have a developmental origin. Particularly maternal nutrition (both under and overnutrition) during gestation has been shown to alter fetal mammary gland development, programming increased breast cancer in adulthood. More recently, experimental studies by our group and others1-3 showed that paternal obesity/high-fat diet consumption or micronutrient deficiency during preconception increased breast carcinogenesis in the female offspring. Although dietary polyphenols are considered promising bioactive compounds in breast cancer prevention, their impact in early development has been less investigated. Here we will present data from our studies that investigated in C57Black mice the effect of maternal and/or paternal consumption of blackberry polyphenols or orange juice effects on breast cancer programming. Results suggest that while maternal (gestation) or paternal (preconception) consumption of blackberry polyphenols reduced the size of mammary adenocarcinomas in female offspring, concomitant maternal and paternal consumption had an opposite effect. In addition, while obese mother (gestation) orange juice consumption protected female mice offspring against breast carcinogenesis, paternal (preconception) consumption had an opposite effect. Altogether, these results suggest that care should be taken when considering intake of high levels of dietary polyphenols during critical developmental periods as increased breast cancer programming could occur depending on the context of consumption.

Keywords: breast cancer, developmental programming, maternal, paternal, dietary polyphenols

Financing: Food Research Center/FAPESP (2013/07914-8)

“Polyphenols from citric modulates brain redox homeostasis: a DOHaD perspective”

Cristiane Matté
Universidade Federal do Rio Grande do Sul, Brazil

Background. Naringin induces neuroprotection and improves adult neurogenesis in different animal models, mainly by reducing oxidative stress and mitochondrial dysfunction. Such evidence, promoted by the media, cause a strong impact on the population’s perception of the consumption of polyphenol-rich supplements. Worryingly, data from pregnant women reports consumption rates of polyphenol-rich supplements of up to 57%. Not surprisingly, several studies concern that high polyphenol intake during gestation may induce metabolic alterations in the offspring. The objective of the present work was to evaluate if maternal naringin supplementation during pregnancy could induce redox and mitochondrial alterations in the offspring’s brain during their postnatal development.

Methods. Pregnant Wistar rats received naringin (100 mg/kg/day, orally) during all or the third week of pregnancy. Offspring’s brain was collected on postnatal days 1, 7, and 21. The study was approved by the ethics commission (N.35332CEUA/UFRGS).

Results. When the dams were supplemented during all the pregnancy, the offspring’s brain was mainly negatively affected. We identify a deficit in the mitochondrial function, as well as a prooxidative profile during the first days of development. On the other hand, when the dams received the naringin during the third week of gestation, our results showed several redox alterations, especially in an antioxidant way, in a sex and region-specific manner in the offspring’s brain. The mitochondrial alterations found in the pups’ cerebellum during the perinatal period were mostly sex dependent. At weaning age, the offspring born from supplemented rats showed increased mitochondrial functionality, superoxide production and reduced DRP1, suggesting reduced mitochondrial fission. Such alterations were accompanied by augmented sirtuin 3.

Conclusions. Our data highlight the importance of carefully consider whether polyphenol supplementation should be advised, and when, during gestation. Since our findings showed that naringin modulated the redox and mitochondrial signaling, which may consequently interfere in the fetal neurogenesis and neurodevelopment.

Keywords: Keywords. Narinigin, Pregnancy, Brain.

Financing: Funding. PROPESQ/UFRGS and CNPq (Universal 442406/2014-2, INCT 465671/2014-4, PQ2 nº: 304293/2018-0 and 303377/2021-6).

"Aging in germ cells and the possible effect of polyphenols in the offspring´s health"

Marcia Manterola
Universidad de Chile

Aging is a significant risk factor in male fertility and is associated to decreased pregnancy outcome, increased abortion rates and offspring alterations and diseases. Overall, aging disturbs testicular function and decreases the number and quality of sperm. Moreover, sperms from aged males exhibit increased DNA fragmentation, altered methylation patterns and a high rate of de novo mutations, suggesting that the genome and epigenome is severely affected by aging. Aging in spermatogenesis is also associated to an increase in free radicals that leads to an oxidative stress condition in the male germ line. Despite this, little is known about the effects of aging and free radicals in spermatogenesis, particularly in the genome of germ cells.

Our lab has shown that in mice, meiotic and post meiotic cells are particularly sensitive to DNA damage in stages where genomic maintenance and stability are crucial to achieve all meiotic and differentiation events. That is, aging in germ cells is associated to DSBs that are even inherited or newly produced in haploid cells. This triggers DNA repair pathways that interfere with transcriptional and recombinational processes that normally occur in meiotic and post meiotic cells. Thus, aging impairs genome stability and the developmental programing during meiosis and spermiogenesis.

In this scenario, polyphenols are promising compounds as they alleviate age-associated cellular damage induced via free radicals. Indeed, resveratrol and curcumin have proven effects in male fertility by improving spermatogenesis and sperm parameters. Thus, by using polyphenols, a key window of opportunity may be originated to establish interventions focused on male reproductive aging and in so doing, prevent diseases in the offspring that can be originated in very early life.

Keywords: aging, male reproduction, spermatogenesis, germ cells, dohad, polyphenols

Financing: Fondecyt 11181329

Symposium 6: Advances in reproductive Cancer Research

Chair: Héctor Contreras

"The current state of reproductive cancers in Chile: CeCan FONDAP Strategic Project"

Héctor Contreras
Universidad de Chile, Chile

In Chile, cancer incidence and mortality have increased steadily, becoming the leading cause of death. Cancers of reproductive tissues such as breast (20.9% women), prostate (28.3% men) and ovary (3% women) affect about 28% of the total population. In these cancers, studies are currently focused on some cellular and molecular aspects, the development of the tumor environment and the establishment of genomic profiles to improve diagnosis, prognosis and treatment. In this symposium, study models to propose therapeutic strategies for recurrence, resistance and metastasis in prostate cancer are presented. All these processes are cross-linked through the CSC-EMT-MET axis for both the dissemination of tumor cells and the subsequent establishment of metastasis. On the other hand, endothelial organization through new blood vessels in tumors is a central aspect. Here we describe cellular mechanisms with therapeutic potential of this vascular network using ovarian cancer as a model. Finally, targeted therapies have emerged as alternatives for breast and ovarian cancer patients in recent years. The success of these therapies lies in the presence of specific molecular alterations. Results of the genomic analysis of breast and ovarian tumors in Chile, which has allowed the identification and characterization of mutations that predict response to therapies and genetic susceptibility to these diseases are presented.

Funding: ANID, Fondecyt 1201407 and FONDAP CECAN 152220002.

"Stemness/Epithelial-Mesenchymal Transition axis, metastasis and resistance in prostate cancer. Potential therapeutic targets."

Enrique Castellón
Universidad de Chile, Chile

Prostate cancer (PCa) is one of the leading causes of male cancer mortality worldwide. Recurrence, castration resistance and metastasis are the main problems. Cancer stem cells (CSCs) are closely related to these processes. The CSC phenotype is maintained by a set of pluripotency genes. Knocking down these genes can reverse CSC characteristics, making them more sensitive to treatments. The Epithelial-Mesenchymal Transition (EMT) has a critical effect on recurrence, metastasis, and resistance as well as on tumor cell plasticity and CSC generation. The objective of this work is to characterize the role of stemness and EMT genes in the progression of metastasis and resistance to castration in PCa and to identify possible new therapeutic targets.

Primary PCa cultures derived from tumor samples and PCa cell lines were used. All the protocols were approved by the institutional Ethics Committees. Cells were cultured under standard and non-adherent conditions (CSC sphere formation). The SOX2, KLF4, and c-MYC pluripotency genes and the EMT genes ZEB1 and SNAIL1 were knocked out with specific shRNAs within lentiviral vectors. The effects of gene knock down on the proliferation, apoptosis, drug resistance, clonogenesis, migration and invasion were analyzed. In addition, the metastasis capacity of SOX2-knocked CSCs was tested in an orthotopic NOD/SCID mouse model.

Stemness gene knocking down showed sensitization to chemotherapeutic drugs, increased apoptosis rate, and decreased clonogenic and invasive capacities. SNAIL1 silencing promoted an epithelial-like phenotype with reduced migration and invasion. SOX2-knocked down CSCs reduced the tumor growth rate and completely inhibited metastasis. ZEB1 silencing sensitized cells to docetaxel and reversed the stemness phenotype. In addition, miRNAs from CSCs exosomes and mesenchymal cells were differentially expressed and seem to be related to the preparation of the pre-metastatic niche in bone. The CSC and EMT genes analyzed have a relevant role in the maintenance of the stemness signature, promoting antiapoptotic, invasive, resistance, clonogenic, tumorigenic and metastatic characteristics. SOX2 and ZEB1 seem to play a determining role in metastatic progression and could be considered as a suitable therapeutic target for PCa.

Funding: ANID, Fondecyt 1201407 and FONDAP CECAN 152220002.

"Elucidation of a tumor cell-derived irrigation system in cancers of the reproductive tissue"

Gareth Owen
Pontificia Universidad Católica de Chile, Chile

Vasculogenic mimicry (VM) is a process by which cancer cells establish an alternative irrigation pathway in an endothelial cell-free manner. This presence of this process in tumor samples possesses a strong correlation with poor patient prognosis. The process of VM *in vitro* can only occur in a 3D matrix. To elucidate the process of VM it is necessary to understand the interaction between the cancer cell and the extracellular matrix. Using an established *in vitro* Matrigel-based model of VM in ovarian and breast cancer cell lines, the gene silencing of integrin β1 (but not β3) is sufficient to prevent VM formation. Protein markers of EMT and stemness show changes in expression and intracellular localization during the first phase of VM formation. RNA-Seq and phospho-array analysis reveal potential novel players in the process of VM. As this pathophysiological phenomenon is strongly associated with reduced patient survival, understanding the mechanisms of VM formation may offer new druggable anticancer targets.

Acknowledgments: FONDECYT 1180241, ANID PIA192015, CONICYT FONDAP-15130011, ANID FONDAP CECAN 152220002. ICM-MINECON IMII P09/16F, Beca ANID Doctorado 21230827 & 21221337

"Genomic analysis of breast and ovarian tumors in Chile reveals new mutations that predict response to therapies and genetic susceptibility to these diseases."

Katherine Marcelain
Universidad de Chile, Chile

Genomic characterization of solid tumors is helping to make cancer a treatable disease. This is because knowing the tumor's mutations can help doctors to identify better and more precise therapies for each patient. Unfortunately, this kind of information is lacking in Chilean patients with solid tumors, including ovarian and breast cancer. To address this gap, we conducted a study using Next Generation Sequencing (NGS) to analyze the presence of driver mutations and mutations that predict response to therapies and genetic susceptibility in ovarian (n=69) and breast (n=75) Chilean cancer patients.

Our results showed that germline and somatic mutations were present in a number of genes, including BRCA1, BRCA2, PTEN, ATM, and TP53, PIK3CA and ARID1A. Additionally, we detected mutations (somatic and putative germline) in TP53, BRCA1, BRCA2, and other genes that were not described in the databases consulted. A high percentage of these variants are potentially driver or pathogenic. This suggests that there may be a number of new mutations that are relevant to the pathogenesis and treatment of cancer in Chilean patients. These findings highlight the need for more genomic research in Chile to better understand the genetic basis of cancer in this population. This information could be used to develop more personalized therapies for Chilean patients with cancer.

Financial Support: ANID FONDEF-ID21I10355, ACT210079, FONDEF IT16I10051. FONDAP CECAN 152220002

Symposium 7: Primary Testicular Failure: Genetic and Hormonal Factors

Chair: M. Andrea Castro Gálvez

"Hormonal dysfunction in patients with primary spermatogenic failure."

Alexis Parada Bustamante
Universidad de Chile, Chile

Infertility affects 13-15% of couples worldwide and in 30% of the cases the source is a male factor. Primary spermatogenic failure, defined as an intrinsic testicular failure in the spermatogenic process, is one of the most common diagnoses in male infertility, but in almost 50% of the cases the etiology remains unknown.

A common characteristic found in men with primary spermatogenic failure is an altered production/ratio of hormones responsible by a correct testicular function, including decreased serum testosterone (T) levels, decreased T/LH, increased 17β-estradiol (E2) and E2/T ratio. In fact, an important percentage of men with this condition have hyperplasia of Leydig cells. However, the mechanisms by which these alterations occur in these men has not been elucidated.

In the last years, our laboratory has showed that these hormonal alterations are related with changes in testicular expression and/or activity of steroidogenic enzymes responsible to produce these hormones, such as CYP19A1 (aromatase) and CYP17A1. Moreover, men with primary spermatogenic failure have an increased testicular expression of steroid sulfatase enzyme, which altogether would increase intratesticular estradiol bioavailability. On the other hand, the mechanisms by which increased estradiol intratesticular concentrations negatively affect testicular function may be related with changes in local production of estradiol metabolites 2-hydroxyestradiol (2OHE2) and 2-methoxyestradiol (2ME2) from estradiol, since men with primary spermatogenic failure have increased testicular expression of enzymes CYP1A1 or S-COMT, responsible to generate these metabolites. Moreover, 2OHE2 blocks testosterone-mediated androgen receptor-dependent gene expression in Sertoli cells in vitro.

In summary, men with primary spermatogenic failure have alterations in testicular production of hormones, mainly estradiol, by changes in the expression/activity of key steroidogenic enzymes. The mechanisms by which an increased E2 intratesticular concentration may affect spermatogenesis process and/or somatic testicular cells could involve an altered production of estradiol metabolites 2OHE2 and/or 2ME2.

Keywords: male infertility estradiol leydig cells

Acknowledgments: Special grateful to all men who accepted participate in our studies and all members of Unit of Reproductive molecular andrology of IDIMI

"The Y Chromosome in infertility and non-reproductive pathology."

M. Andrea Castro Gálvez
Universidad de Chile, Chile

The Y chromosome has a widely recognized role in spermatogenesis and sex determination. Events of intra-chromosomal recombination are the basis for the maintenance of their non-recombinant male specific region (MSY), but also lead to variations of genetic content in the AZF regions. Microdeletions of the long arm of Y chromosome (Y-MD) is the best known genetic cause of male infertility. They are found in 10%–15% of men with primary spermatogenic failure and involve 1-3 AZF regions. In addition, partial-AZFc deletions have been shown to increase the risk of infertility, germ cell testicular tumors and may be more prevalent in certain lineages of the Y chromosome. More recently, copy number variations (CNVs) with loss or gain of pseudoautosomal and MSY genes in subjects with AZFb+c terminal deletions have been associated with abnormalities in growth and neuropsychiatric function. Dosage-sensitive regulatory X-Y gene pairs in MSY contribute to differences between XX and XY individuals beyond the reproductive system. They may play important roles in neuronal development, cardiovascular function and male-bias in several diseases, including cancers. This concept is reinforced by the increased incidence of age-associated diseases in men with mosaic loss of the Y chromosome. Accordingly, there are significant transcript levels of genes in AZFa (USP9Y, DDX3Y, UTY) and AZFb (HSFY, KDM5D, EIF1AY), or between those regions (NLGN4Y) in many tissues. Among these, DDX3Y, encodes a DEAD-box RNA helicase critical for neuronal differentiation, where other MSY-genes are upregulated in mature neurons (RPS4Y, ZFY, SRY, EIF1AY and PRY). Similarly, EIF1AY encodes a translation initiation factor with an increased transcriptional and protein expression in the heart of healthy men. Therefore, in order to understand the possible roles of Y chromosome-CNVs in infertile patients, their clinical evaluation and follow–up must be extended beyond the infertility field.

Keywords: Y chromosome, pseudoautosomal and MSY genes, AZF regions, male infertility, spermanogenic failure, non-reproductive diseases

Financing: Fondecyt 1030984; 1120176, University of Chile.

Acknowledgments: Andrology team of the IDIMI; Patients and their families; Organizing and Scientific Commitee - Valdivia 2023

"Gene variants of the RAS/MAPK pathway and androgen receptor polymorphisms in the etiology of cryptorchidism and primary testicular infertility."

Fernando Adrián Rodríguez Convertino
Universidad de Chile, Chile

The incomplete descent of testis through the inguinal canal into the scrotum, known as cryptorchidism, is observed in approximately 1–9% of boys at birth. The persistence of cryptorchidism represents a risk factor associated with testicular cancer and subfertility. The influence of different factors over testis descent has been extensively studied, including genetic factors. Nevertheless, the etiology is unknown in most cases and consequently, its impact over fertility could be heterogeneous.

A group of syndromes caused by germline mutations in genes that encode components of the Ras/mitogen-activated protein kinase (MAPK) pathway (Ras/MAPK), known as “RASopathies”, present cryptorchidism as a highly frequent feature (60–77%). We studied a cohort of 239 patients with isolated cryptorchidism and detected five nucleotide substitutions (SOS1_p.R497Q, BRAF_p.F595L, NRAS_p.T50I and MAP2K2_ p.Y134C) in 2.9% of the patients. This incidence is similar to that reported for INSL3/RXFP2 variants (2.3%) in 303 boys with persistent cryptorchidism.

On the other hand, the role of the Androgen Receptor (AR) is particularly relevant at the last phase of testicular descendent (inguinoscrotal phase). The AR present at the transactivation domain two polymorphic trinucleotide repeats (CAG and GGN) and, consequently, genomic response to androgens may be affected by these polymorphisms. In a cohort of 109 patients with inguinal cryptorchidism we found a significantly longer CAG repeats in bilateral cases than in control (73% vs. 49%; p=0.032) and a higher risk of bilateral cryptorchidism (OR=4.05, 95% CI 1.71-9.62) for the combination CAG>22/GGN=23 repeats.

The Ethics Committees of School of Medicine, University of Chile and the Central Metropolitan Health Service in Santiago, Chile approved these studies.

Although these and previous genetics findings shed light over cryptorchidism etiology, it seems that this congenital alteration is a consequence of genetics, environmental and maternal factors. Consequently, further efforts to integrate and determine the specific weigh of these factors should be carry out.

Keywords: cryptorchidism; RAS/MAPK pathway, androgen receptor, CAG repeat polymporphism

Financing: Fondecyt 1060081 and 1140450

Acknowledgments: We are grateful to the patients and their families for helping us to perform these studies.

"Genetic variants of LIN28B: association with seminal quality and testicular function in adult males."

María Cecilia Lardone
Universidad de Chile, Chile

Since the complexity of the spermatogenic process in terms of the genes involved, it is tempting to speculate that genetic polymorphisms may influence spermatogenesis. In this sense, genome-wide association studies are continually identifying new variants and imputing genes associated with spermatogenic failure, which require further replication in other populations or functional verification. Additionally, there is a significant trend to common single nucleotide polymorphisms (SNPs) occurring in mild forms of spermatogenic failure.

Recently, our and other large-scale genomic studies have identified genetic variants in LIN28B to be robustly associated with pubertal traits such as age at gonadarche and pubertal Tanner Stage in men. The LIN28-genes are known to control cell division, growth, and differentiation, through the interaction with let-7 microRNAs. Consistent with its association with puberty, Lin28b is expressed in hypothalamus, pituitary, and the gonads in pre-pubertal and post-pubertal animals. Conversely, in adults, LIN28B is exclusively expressed in the testicular germ cells and placenta. Furthermore, two studies have shown that self-reported later onset of puberty is associated with reduced sperm concentration, total sperm count and testosterone levels, suggesting that age of puberty may influence male reproductive function later in life, possibly involving a common genetic factor.

Our laboratory found a significant higher frequency of LIN28B genetic variants (rs7759938_C, rs314280_T, rs395962_T and rs314268_A in infertile men with low sperm count suggesting that common LIN28B gene variation confers susceptibility to oligozoospermia. Moreover, the same genetic variants are associated with reduced LIN28B transcriptional expression in sperm cells in these subjects, implying that these genetic variants are expression quantitative traits locus. The vast amount of data emerging from the large-scale studies gives us the opportunity to uncover novel loci, their protein families and biological function involved in causes of spermatogenic failure to find an explanation for more than 60% of unknown male infertility cases.

Keywords: common genetic variants, LIN28B, oligozoospermia, genetic causes of male infetility

Financing: FONDECYT#11200898

Symposium 8: Risk of Reproductive Disorders by Toxic Agents

Chair: Fernanda Parborell

"Effects of the herbicide glyphosate on the testicular function."

María Fernanda Riera
Hospital de Niños Ricardo Gutiérrez, Argentina

Sertoli cells (SC) play a central role in the development of a functional testis. The quantity of SC obtained during proliferative periods determines daily sperm production. In addition, SC functions, such as germ cell nourishment and blood-testis barrier (BTB) integrity, are essential to maintain spermatogenesis.

Glyphosate (G)-based herbicides are used worldwide in agriculture and weed control. Roundup (R) is the most widely used formulation. Several studies showed that G or R have adverse effects on male reproduction. However, most of them used doses far above the maximum environmental exposure levels reported in humans. Then, whether G or R are harmful to male reproductive health when exposure occurs at low doses and at early life stage is still under debate. Thus, we proposed to analyze the effects of low doses of G or R on proliferation and differentiated SC functions. We performed in vitro analysis using SC cultures and in vivo experiments in SD rats. Experimental design in animals was approved by the Local Institutional Ethical Committee (CICUAL-Res#2018-02). We observed that R decreased SC proliferation in vitro but not in in vivo experiments. Regarding differentiated SC functions, studies in vitro showed that neither G nor R caused impairment in lactate production. However, G and R decreased Transepithelial Electrical Resistance and promoted claudin 11 delocalization, suggesting a disruption in junctions' assembly. Also, we observed a significant increase in BTB permeability and disorganized seminiferous epithelium in 2 and 50 mg/kg/day G- or R-treated juvenile animals. However, G or R-treated juvenile animals displayed normal BTB permeability and spermatogenesis in adulthood.

In summary, continuous exposure to low doses of G or R alters BTB permeability in juvenile rats. However, considering that adult animals treated during the juvenile stage showed no differences in spermatogenesis, it is feasible to think that BTB impairment is a reversible phenomenon.

Keywords: Sertoli cells, Glyphosate, Blood-testis barrier.

Financing: PICT 2020-1061, PIP 2020-0162, PICT 2021-CATII-00027

"Impact of the uv filter benzophenone-3 on reproduction: lessons from in vivo and in vitro models."

Horacio Rodríguez
Universidad Nacional del Litoral (UNL), Argentina

Benzophenone-3 (BP3) is an UV filter commonly used in sunscreens. We studied if some critical processes for reproduction can be identified as targets of BP3 action. We used two exposure schemes in pregnant C57Bl/6 mice: early prenatal and perinatal exposure. Besides, we performed the scratch assay with Swan71 cells as well as we tested BP3 action on an in vitro model of implantation. Protocols were designed in accordance with the Guide for the Care and Use of Laboratory Animals issued by the U.S. National Academy of Sciences and approved by the Ethical Committee of our institution (CE 2018-62). We showed that pregnant mice exposed to BP3 have smaller whole implantation sites (WIS) and fetus-placental index (FPI), leading to fetal growth restriction. These growth abnormalities were linked to an impaired spiral artery remodeling (SAR) with reduction in NK cells. We found that BP3 reduced the migration ability of Swan 71 trophoblast cells, which restored to normal values when cells were exposed to BP3+flutamide, an AR inhibitor. Besides, a significant delayed hatching and attachment of the BP3-treated embryos was observed, and a reduction of implantation, demonstrating a direct action of BP3 on in vitro early embryo development. We also studied if BP3 can affect ovulation rate. We observed that prenatal exposure in two successive pregnancies to BP3, reduced the number of ovulated oocytes. These results showed that early prenatal period is a sensitive period to dermal exposure to BP3. Then, we asked if BP3 could exert detrimental effects through a perinatal exposure to BP-3 comprising gestation and breastfeeding. We observed a decrease of fertility linked to oocyte depletion in the offspring born to mothers perinatally exposed to BP3 in a Fertility Assessment by Continuous Breeding protocol (FACB). We conclude that BP3 can affect different reproductive processes considered critical for an optimal fertility.

Keywords: BENZOPHENONE 3, UV FILTER, SUNSCREENS, FERTILITY, OVARY, EMBRYOS, FETAL GROWTH

Financing: CONICETUNLANPCyT (PICT 2018-03617; PICT2020-00897)

"Exposure to endocrine disruptors chemicals and risk of endometriosis."

Florencia Chiappini
Universidad de Buenos Aires, Argentina

Background: Endometriosis is an estrogen dependent gynecologic disease, defined by the presence of stromal and/or endometrial glandular epithelium implants in extra-uterine locations. The etiology of endometriosis remains uncertain. Several studies support the fact that detached endometrial tissues of menstruation reach the peritoneum by retrograde movement to get implanted, followed by acquisition of new blood supply through angiogenesis, and grow developing endometriotic lesions. Humans are daily exposed to chemical pollutants that could adversely influence physiological processes and cause diseases, including endometriosis. Growing evidence suggests that endocrine disrupting chemicals (EDCs) may be etiologically involved in the development of disease. EDCs can mimic hormones altering signaling pathways. Hexachlorobenzene (HCB), an organochlorine pesticide, is a weak ligand of the Aromatic Hydrocarbon Receptor (AhR) and acts as EDC.

Methods: 1) in vivo endometriosis rat model, 2) in vitro models: a) human endometrial cell line b) primary cultures of endometrial stromal cells from eutopic endometrium of control and women with endometriosis. This study was approved by the Ethics and Research Committee from the Biology and Experimental Medicine Institute (IBYME-CONICET) of Buenos Aires, Argentina.

Results: Our results showed, in rat endometriosis model, that HCB increases endometriotic like-lesions volume, microvessel density and the vascular endothelial growth factor (VEGF), cyclooxygenase-2 (COX-2), AhR and Aromatase expression levels. Moreover, disrupts hormonal receptors expression and increases tumor necrosis factor-α (TNF-α) content in peritoneal fluid. In in vitro models, we observed that HCB enhances cell migration and invasion, and matrix metalloproteinases (MMP-2-9) activities. Moreover, the pesticide promotes prostaglandin E2 (PGE2) and VEGF secretion and c-SRC kinase activation. In addition, the pesticide enhances the angiogenesis process inducing endothelial cells proliferation, migration and their capacity to tube formation.

Conclusions: Our results provide experimental evidence that HCB induces alterations associated with endometriosis, suggesting that these mechanisms could contribute to EDCs exposure-induced endometriosis development.

Keywords: Organochlorine pesticide, Aryl hydrocarbon receptor, Endocrine Disruptors, Endometriosis

Financing: National Council of Scientific and Technological Research, CONICET, Argentina; University of Buenos Aires, Argentina; and National Agency of Scientific and Technological Promotion, ANPCyT

"Promising ovarian and uterine protective agents during chemotherapy: State of the art, challenges, and prospects."

Fernanda Parborell
Instituto de Biología y Medicina Experimental, Argentina

The consequences of cancer and its treatment on female reproductive function are becoming increasingly well-documented. Generally, compared to the general population, women have a 38% lower likelihood of achieving pregnancy after undergoing antitumor treatments. With the improved survival rates of both children and reproductive-age patients receiving anti-tumoral regimens, it is imperative to understand the impact of these treatments on the quality of life and explore new techniques focused on fertility preservation.

Chemotherapeutic drugs such as doxorubicin and cyclophosphamide have been found to significantly impair ovarian function by disrupting follicular development and subsequently affecting fertility. The situation is alarming for women who receive antitumor treatments after the age of 20, as the rate of post-treatment amenorrhea reaches 80% and premature ovarian failure (POF) occurs in 90% of cases. POF is a reproductive disorder characterized by the depletion or dysfunction of ovarian follicles in women under the age of 40. The patients present amenorrhea, hypoestrogenism, and elevated levels of gonadotropins.

Currently, the available therapeutic options for preserving fertility in women before initiating antitumor treatment consist of invasive techniques such as cryopreservation of oocytes, embryos, and ovarian cortex, as well as the administration of non-invasive GnRH analogues.

In our laboratory, we employ two chemotherapeutic drugs, doxorubicin (DOXO) and cyclophosphamide (CTX), to induce premature ovarian failure in a mouse model. This enables us to investigate the impact on female gonads when patients undergo antitumor treatments. Given the lack of effective, affordable, and non-invasive strategies, we propose melatonin and resveratrol as potential "ovarian protector agents" to be administered before or during these treatments, aiming to preserve female fertility. It is crucial to note that any compounds under consideration for evaluation as potential candidates to protect the ovary and preserve fertility in cancer patients must not interfere with the antitumor effects of the aforementioned treatments.

Keywords: Oncofertility, Ovary, Ovarian reserve, Apoptosis, Angiogenesis, Oxidative stress, steroidogenesis

Financing: National Agency for Scientific and Technological Promotion (PICT 1603–2017) National Cancer Institute (INC 2023-2024) Baron and Williams Foundations

Acknowledgments: This study was supported by National Agency for Scientific and Technological Promotion, National Cancer Institute and Innovation and Baron and Williams Foundations, Argentina.

Symposium 9: The Impact of Breastfeeding on Infant Health

Chairs: Reyna Peñailillo and Susana Contreras

"The impact of health policies and COVID-19 on exclusive breastfeeding: an interrupted time series analysis at the national level"

Deborah Navarro Rosenblatt
Subsecretaría de Salud Pública, Chile

Background: In 2011, Chile added 12 mandatory weeks of maternity leave (ML). In January 2015, a pay-for-performance (P4P) strategy was introduced in the primary healthcare system, incorporating exclusive breastfeeding (EBF) promotion actions. COVID-19 led to healthcare access difficulties. The aim of this study was to evaluate the effect of the extended ML, the P4P, and COVID-19 on EBF prevalence and inequalities, at 3 and 6 months in Chile.

Methods: Aggregated EBF prevalence data from public healthcare users nationwide (80% of the Chilean population) was collected by month. Interrupted time series analyses were used to quantify changes in EBF trends from 2009 to 2020. EBF heterogeneity was assessed by urban/rural and geographic settings. We assess the impact in EBF inequalities using two procedures: 1. ITSA stratified by municipal SES quintiles (Q1-Q5); 2. EBF slope index of inequality (SII).

Results: We found no effect of the extended ML on EBF; the P4P strategy increased EBF at 3 months by 3.1% and by 5.7% at 6 months. COVID-19 reduced EBF at 3 months by -4.5%. Geographical heterogeneity in EBF was identified. The P4P strategy increased EBF at 6 months in all SES quintiles, but in a higher level in poorer municipalities (SII: -0.36% and -1.05%). During COVID-19, wealthier municipalities showed a slightly higher EBF prevalence at 6 months (SII: 1.44%).

Conclusion: The null effect of ML on EBF in the public healthcare system could be explained by low access from public healthcare users to ML (20% had access to ML) and by an insufficient ML duration (5.5 months). The P4P strategy includes multiple interventions that seemed effective in increasing EBF across all SES quintiles, but further in lower quintiles. The negative impact of COVID-19 on EBF should alert policy makers about the crisis's effect on health promotion activities, especially in poorer municipalities.

Keywords: Exclusive breastfeeding; health policy; inequalities.

Financing: This work was supported by the Chilean FONDECYT, under grants 1130277 and 1150878. DN-R held a PhD fellowship from the Commission of Science and Technology, under grant 21151097

Acknowledgments: We thank the Social Welfare Superintendence (SUSESO) for providing data on ML access and the Ministry of Health for the data on EBF. Special thanks to Benaiah and to Sarah

“Determination of biomarkers for postpartum depression in breast milk”

Reyna Peñailillo
Universidad de los Andes, Chile

Background. Postpartum depression (PPD) is the most common complication associated with childbirth and exerts harmful effects on children. This project aims to identify neurogenesis-associated microRNAs present in extracellular vesicles (EVs) in the breast milk of mothers with PPD.

Methods. Milk samples were collected between 1 and 6 months postpartum. Each mother completed the Edinburgh PPD survey and donated between 20-50ml of breastmilk in the early morning. Milk samples were centrifuged, defatted, and filtered. EVs were isolated by ultracentrifugation, pelletized, and resuspended in PBS. Size distribution and particle concentration were analyzed by the Nanosight system; meanwhile, the presence of exosomal markers CD9, CD63, and CD81 were analyzed by flow cytometry. RNA from EVs was isolated, and cDNA synthesis was performed to determine the expression of miRNAs.This study was approved by the Ethical Scientific Committee of Universidad de los Andes (CEC2021094)

Results. Fourteen mothers completed surveys and donated breastmilk at 3.4 ± 1.7 months postpartum. Depression group scored 14.8 ± 2.5 pts in Edinburgh survey; meanwhile, the control group scored 4.3 ± 1.3 pts. EVs showed the presence of exosomal markers CD9, CD3, and CD81. Neurogenesis-associated microRNA mir-200a-3p showed an increased expression in breast milk from mothers with PPD ($p=0.04$).

Conclusions. Breastmilk from mothers with PPD showed an increased expression of miRNA associated with neurogenesis, demonstrating that breast milk can be a source of biomarkers for PPD and can be transferred to the new born.

Keywords: Breastmilk, post-partum depression, extracellular vesicles

Financing: Fondo de Desarrollo Científico SOCHINUT- Henri Nestlé 2021; ANID-BASAL funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024

“Effect of obesity on the profile of fatty acids in breast milk”

Cynthia Barrera Ramírez
Universidad de Chile, Chile

Background. The lipid fraction of breast milk (BM) plays a fundamental role in the development of the newborn1, providing essential fatty acids (AG), necessary for proper brain and visual development. Depends on its maternal supply through breast milk itself, which in turn can see its content modified depending on the mother's diet2. Obesity currently affects more than 60% of pregnant women. Although obesity has been shown to cause alterations in FA metabolism, few studies have specifically evaluated the effect of maternal obesity on the fat content of BM. To determine the effect of maternal obesity on the concentration of fatty acids in the milk of mothers between the first and second month of lactation.

Methods. Normal weight (NW=20) and obese (OB=20) women between one and two months of exclusive breastfeeding were recruited. Diet was evaluated through a food frequency questionnaire; weight and height measurement, and extraction of a milk sample were carried out, which was stored at -80°C until analyzed. The determination of the fatty acid profile was determined by gas-liquid chromatography, expressed as methylated fatty acids per 100 ml of sample. Data were analyzed by Mann-Whitney t-test and considered significant when $p<0.05$.

Results. Although clearly lower diet quality was observed in the OB group, the total intake of n-6 and n-3 polyunsaturated fatty acids (PUFA), ALA, EPA, and DHA were similar between the groups. In BM, the AA and DHA concentration was lower in the OB group.

Conclusions. PUFA n-3 and AA and DHA levels were reduced in BM in obese women. It is necessary to develop a strategy to increase n-3 PUFAs during the lactation period, particularly in women with obesity.

Keywords: Maternal obesity, fatty acids, breast milk.

Financing: Premio SOCHINUT-Henri-Nestlé, 2021.

“Non-nutritive sweeteners: presence in breast milk and impact on infant health”

Martín Gotteland
Instituto de Nutrición y Tecnología de los Alimentos, Chile

Over the last decade, the use of NNS to replace sucrose in foods has increased considerably worldwide, and more particularly in Chile where the proportion of foods with NNS (55.5%) is now much higher than in other countries. For Chilean consumers, it is becoming increasingly difficult to find foods that do not contain ENNs, in a context where the health effects of these additives are highly controversial. Some studies in the US indicate that ENNs consumed by the mother can pass into the amniotic fluid and breast milk, exposing the foetus and infant to ENNs without knowing the impact of such exposure on infant health. It has been postulated that infants exposed early and chronically to increased sweetness may have an increased sweet taste threshold, favouring later consumption of sweeter foods. In a recent study of 260 pregnant women in Santiago, we observed that all of them consumed ENNs, mainly aspartame, sucralose, and acesulfame-K. Of 126 milk samples obtained from these mothers, 10.3% contained sucralose, which was present in concentrations < 10ng/ml, i.e. below its detection threshold (200ng/ml), suggesting that it should not be detected by the infant. Determination of the sweet taste threshold of a subgroup of these infants after weaning showed that this parameter did not vary as a function of maternal ENN consumption or infant weight. Although these results seem to rule out a negative effect of maternal ENN consumption on infants, recent studies indicate that certain ENNs exert biological activities at concentrations lower than those detected by sweet taste receptors. Further studies are therefore needed to determine whether ENNs present in breast milk are truly inert (Fonis SA18I0062).

Symposium 10: Insights into early life programming in the offspring of women with obesity

Chairs: Erika Castaño-Moreno and Paola Casanello

"Short and long-term preterm infant health in the double burden of maternal malnutrition"

Fabiola Suano de Souza
Departamento de Pediatria da Universidade Federal de São Paulo - Escola Paulista de Medicina, Sao Paulo, Brazil

Brazil is an unequal and developing country where 30% of pregnant women are less than 20 years old, and 11% of live births are preterm. The principal causes of prematurity are maternal diseases (genitourinary infections, hypertension, diabetes, and congenital malformations). In the country, there is a Public and Universal Health System (SUS) that promotes the care for preterm infants and their mothers through policies such as Child-Friendly Hospitals, Kangaroo Method, and Human Milk Banks. Women of childbearing age, pregnant, and children have combined nutritional problems such as iron deficiency anemia, vitamin A deficiency, and obesity. According to these characteristics, which are different from developed countries, it becomes important to study better the effects of early nutrition and postnatal growth on developmental, health, and long-term disease risk in preterm infants, especially in those with very low weight. In the last 5 years, I have developed cross-sectional and cohort studies that evaluate the feeding practices (breastfeeding, complementary feeding), postnatal growth, and development, as well as risk factors for cardiovascular diseases in preterm children. The main findings of these studies were: a high percentage of small for gestational age preterm infants, the relationship between vitamin D deficiency in pregnancy and prematurity, breastfeeding practices associated with better catch-up growth using Intergrowth-21 curves, normal renal function, and blood pressure levels, and worst lipid levels and markers for cardiovascular disease in preterm children up to 8 years of age. Presenting these findings and comparing them with data from other developed countries can bring new opportunities for long-term health promotion and disease prevention strategies in preterm children.

Keywords: Preterm birth; nutrition; SGA; Vitamin D; breastfeeding

“Insights into developmental mechanism of early programming of obesity in the offspring of women with gestational obesity”

Paola Casanello
Dept de Neonatología y Dept Obstetricia, Pontificia Universidad Católica de Chile, Santiago, Chile

Maternal obesity is the most relevant risk factor for developing childhood obesity and metabolic syndrome in the offspring. The mother’s inflammatory and metabolic status can be transferred to the developing embryo and fetus, which can program cell metabolism. In this presentation, a short review of the mechanisms by which maternal obesity can alter the fetal immune function, fetal adiposity and placental function will be described. We set the study at birth and follow-up (4 months) of the offspring of women with pregestational obesity who participated in a randomized controlled trial of docosahexaenoic acid (DHA) supplementation during pregnancy. The results of this follow-up on neonatal body composition, immune function and methylome, metabolic markers (lipid profile, HOMA-IR, leptin, adiponectin) and placental markers of inflammation and fatty acid transporters will be presented and discussed. The results presented will shed light on our current research interest, where we study the possible mechanism of how the obesogenic intrauterine environment could alter the offspring´s progenitor hematopoietic and mesenchymal stem cells, compromising their early cell commitment. These molecular changes are critical to the infant´s programming of the risk of obesity and chronic diseases and are fundamental to understanding how and when prevention needs to start.

Keywords: Maternal Obesity; Inflammation; immune cells; adipogenesis; DOHaD

Funding: FONDECYT #1171407 and #1221812- ANID, Chile

“Targeted Metabolomic to understand the metabolic alterations related to maternal obesity”

Erika Castaño-Moreno
Institute for Obesity Research, Tecnológico de Monterrey, México

In the field of Developmental Origins of Health and Disease (DOHaD), maternal obesity significantly impacts not only a mother's health but also a developing fetus and the child's postnatal well-being. Extensive epidemiological studies have explored the multifaceted risks linked to pregestational obesity, encompassing heightened maternal weight gain, gestational diabetes, pre-eclampsia, fetal anomalies, excessive fetal weight, stillbirth, increased childhood obesity, and metabolic syndrome.

Nevertheless, a critical aspect that remains elusive is the intricate association between maternal obesity and the metabolism of vitamins and fatty acids. Investigating this connection poses challenges due to complex, costly, and limited-access methodologies not universally available in research labs.

Assessing complex B vitamins in pregnant women with pregestational obesity and their newborns reveals lower levels of vitamin B12 and various folate forms. Women with pregestational obesity exhibit lower folate status than normal-weight counterparts and lower vitamin B12 compared to reference values. Intriguingly, mothers carrying female fetuses display diminished folate and vitamin B12 levels compared to those with male fetuses. Furthermore, female newborns have reduced folate and vitamin B12 status relative to males. Notably, 5,10-methenylTHF, a vital marker for fetal growth and development, exhibited associations with multiple fetal growth variables in maternal and cord blood.

In a study of 7 to 17-year-old children, subjects were categorized into three groups: type 2 diabetes mellitus (T2DM), metabolic syndrome (MS), and a control group. Using a lipidomic approach to investigate the relationship between lipid fractions and fatty acids bound to triglycerides, cholesterol, and phospholipids. Plasma lipid levels and fatty acid fractions displayed variances among the groups, with specific fatty acids associated with inflammation markers in both the MS and T2DM groups.

Utilizing metabolomics and lipidomics is pivotal in comprehending metabolic alterations in conditions like maternal obesity, childhood obesity, and childhood diabetes, revealing shifts in micronutrient metabolism and fatty acid composition.

Keywords: metabolomics, maternal and childhood obesity, B vitamins

"Maternal B-12 concentrations and alterations in glucose metabolism: findings of the CHIMINCs-II study"

Fernanda Mujica

Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile

Background. Evidence has shown that maternal B-12 deficiency is associated with a higher risk of gestational diabetes (GD) and insulin resistance (IR) in the mother 1,2. The COVID-19 pandemic has compromised micronutrient intake such as B-12, which may increase the risk of the double burden of malnutrition 3, especially in countries with a high prevalence of obesity such as Chile.

We sought to explore factors related to B-12 status and the relationship between B-12 status and alterations in glucose metabolism in Chilean pregnant women.

Methods. This study is nested within the CHIMINCs-II cohort; includes 413 pregnant women enrolled in Santiago, Chile (2020-2022). Maternal blood samples and lifestyle information were collected at early pregnancy. B-12 was measured by immunoassay. Indicators of alterations in glucose metabolism were measured as follows: insulin and HbA1c (%) by immunoassay and HPLC, respectively. We accessed glycemia and GD information from clinical records. Low B-12 and deficiency were defined as B-12 <221-pmol/L and <148-pmol/L, respectively. IR was defined as HOMA ³2.5 and HbA1c >5.7% was considered as altered.

Results. Median (IQR) B-12 concentration was 169 (125-231)-pmol/L; 37% of women were B-12 deficient and 70% had low B-12. Overall, >40% had obesity, 20% had GD and 46% had IR. B-12 deficient women had higher pre-pregnancy BMI, lower education, and use of prenatal multivitamin supplementation, compared to non-deficient. B-12 concentration was related to lower IR chance (OR=0.99, P=0.04; logistic regression); however, this association was not significant after adjustment. No significant association was found between B-12 and glucose-related indicators.

Conclusion. In Chilean pregnant women recruited during the COVID-19 pandemic, we observed a high prevalence of B-12 deficiency, which coexists with a high prevalence of obesity. Despite that B-12 status was not associated with glucose metabolism-related alterations, it is urgent to assess the potential adverse consequences of maternal B-12 deficiency in the offspring.

Key words. Vitamin B-12, pregnant women, deficiency, obesity

Funding. FONDECYT#3210464, FONDECYT#1190532, ANID-COVID#0591

SChRD New members presentations

Chair: Maritza Garrido

"Nutrigenomics of choline in brain angiogenesis"

Nicolás Santander
Universidad de O´Higgins, Rancagua, Chile.

Background. The brain is an energy-intensive organ that depends on a complex network of blood vessels for its normal functioning. Vascular patterns are defined during embryonic development in a stereotyped fashion, failure of which leads to neonatal and adult pathology (blood vessel malformation or blood-brain barrier dysfunction). Understanding the exquisite regulation of brain vascularization is necessary to prevent or modify these pathologies. We have explored the role of metabolic cues in brain angiogenesis, specifically choline, and (although seemingly unrelated) cholesterol.

Methods. All studies were approved by insitutional ethics review boards. To study choline role in brain vascularization, we generated a mouse mutant and cells lacking *Flvcr2*, a choline importer expressed almost exclusively in brain endothelial cells. For the evaluation of the role of cholesterol, we use cells with reduced cholesterol content and embryos lacking the most prominent HDL receptors, SR-B1 and ABCA1, which import and export cholesterol, respectively. General vascular phenotypes were evaluated using histological methods, while nutrigenomic effects were studied with RNA sequencing.

Results. Inactivation of *Flvcr2* in mice led to severe vascular malformations in the neurogenic niche, congenital hydrocephalus, and perinatal death. Transcriptomic analysis of FACS-isolated endothelial cells showed widespread alterations in expression of genes involved in angiogenesis, some of which were confirmed by Western blot and immunofluorescence. Similar effects were observed in cultured cells with CRISPR-mediated inactivation of *Flvcr2.* Importantly, in these models, cholesterol metabolism genes were strongly differentially expressed. Partial removal of cholesterol from cells *in vitro* led to increased migration in a scratch assay and transcriptomic alterations opposite of *Flvcr2* inactivation. Lack of SR-B1 in fetuses was associated with a mild vascular phenotype (the appearance of tortuous vessels) and a weaker transcriptomic signature.

Conclusions. Choline and cholesterol transport are important for vascular morphogenesis in the brain.

Keywords: Embryo development, vascular morphogenesis, cholesterol

Financing: AHA postdoctoral fellowship 20POST35120371, Institutional funds (UCSF, UOH).

“In search of an in vivo model to study vasculogenic mimicry”

Pamela González
Pontifica Universidad Católica de Chile, Santiago, Chile.

Introduction: Vasculogenic mimicry (VM) is a process by which cancer cells establish an alternative perfusion pathway in an endothelial cell-free manner. Although a strong correlation with poor patient survival has been observed, there is still controversy surrounding the validity of current research models. Our laboratory has established and in vitro model of VM, however an in vivo model and a definitive marker of VM in clinical samples remains elusive.

Materials and methods: Cancer cell lines known to undergo VM in vitro were transplanted into the mouse flank. The human ovarian cancer line (HEY-A8) was introduced into immunosuppressed NOD/SCID mice, and the mouse breast cancer line (4T1) was introduced into BalbC mice. Both cancer lines produced tumors that were removed, fixed and cut into histological sections. Ovarian cancer patient samples were obtained with ethical approval from the Biobank at the pontific Universidad Católica de Chile. Human and mouse sections were examined by H&E and PAS staining, and immunofluorescence (IF) and immunohistochemistry technique with CD31 and pan-laminin.

Results: In mouse and human samples, luminal tubular structures were identified with H&E staining, with a PAS+ network around the structure, in addition to pan-Laminin expression and absence of CD31 in cancer cells. IF of CD34 and pan-Laminin in 4T1 tumors and pan-laminin in cancer cell lines suggested luminal structures.

Discussion: Although evidence for luminal tubular structures was observed in both in vivo mouse and human samples, a further protein marker is still required allow the clinical confirmation of VM presence.

Keywords: ovarian cancer, vasculogenic mimicry, xenograft

Financing: Funding: FONDECYT 1220586, ICN09_016/ICN2021_045, CONICYT FONDAP-15130011 and ANID/FONDAP 152220002

“Production of bovine embryos with predetermined paternal genoma by using genotyped haploid androgenetic embryonic cells”

Luis Águila
Universidad de La Frontera, Temuco, Chile

Background: Previous studies in mice and primates have shown that the sperm genome can be replaced by haploid androgenetic embryonic stem cells for the generation of live animals, a method termed ‘semi-cloning’ (SC). Thus, our aim was to determine whether bovine offspring could be derived by SC using haploid androgenetic embryonic cells as the paternal genome.

Methods: Animal handling followed the Canadian Council on Animal Care guidelines for farm animals. This study did not require the handling of animals in the facilities of the University of Montreal and the Universidad de La Frontera. Haploid androgenetic morula-stage embryos were biopsied and genotyped using a GGP 100K Bovine SNP array. Reconstruction of SC zygotes was performed by the injection of a blastomere into the cytoplasm of a parthenogenetically activated oocyte. Reconstructed blastocysts were biopsied and genotyped to confirm paternal contribution and then cryopreserved for embryo transfer. Gestations were stopped at around 100 days of gestation to obtain conceptuses. Semi-quantitative RT-PCR and gene-specific bisulfite sequencing were performed to assess expression patterns of imprinted genes (IGF2, IGF2R, PHLDA2, SNRPN, and KCNQ1OT1) and methylation pattern of the KCNQ1 DMR from different fetus’s tissues.

Results: Cleavage rates in vitro were similar but with delayed development and poor morphology of SC-derived embryos compared to ICSI controls. Genomic analysis confirmed paternal contribution in 72% of the SC embryos. Although fewer recipients transferred with SC blastocyst were pregnant (30%) at day 30 compared to ICSI (100%) ($p=0.07$), the crown-rump length (15.3 and 16.5 mm, respectively) and fetometric measurements were similar, except for a larger volume of amniotic fluid in the SC group. Finally, the expression of imprinted genes and methylation of the KCNQ1 DMR were also similar between groups.

Conclusion: This study shows that genotyped haploid androgenetic embryonic-derived cells can be used for producing cattle with a predetermined paternal genome.

Keywords: early development, in vitro breeding, IVF, nuclear transfer

Financing: FONDECYT 11230091, ANID-CHILE, NSERC-Canada (CRDPJ 536636-18 and CRDPJ 487107-45), SPEC program of FAPESP, Brazil, and Programa de Formacion de Investigadores Postdoctorales (PDT21-0001) Universidad de La Frontera.

Acknowledgments: Servidor de Soroban (SATREPS MACH – JPM/JSA1705) en el Centro de Modelación y Computación Científica de la Universidad de La Frontera.

“Cryopreservation affects viability of bovine MSC depending on tissue source whereas maintaining their intrinsic properties”

Javiera Bahamonde
Universidad Austral de Chile, Valdivia, Chile

Background: The study of mesenchymal stem/stromal cells (MSC) and their therapeutic potential in domestic species has become quite relevant, but in cattle the information available is very limited. Cryopreservation is broadly used to preserve MSC, but little is known about the potential effect of this process on the intrinsic properties of MSC, beyond their survival. Additionally, one of the limitations for the development of MSC-based therapies is the high variability among MSC. It has been proven that even MSC coming from different tissues from the same individual could react differently when faced with certain stimuli, therefore different reactions to cryopreservation depending on the tissue of origin could be expected. The aim of our study was to assess the effect of cryopreservation on viability and intrinsic properties of fetal bovine MSC from bone marrow (BM-MSC), adipose tissue (AT-MSC) and placenta (PT-MSC).

Methods: MSC were obtained from abattoir-derived fetuses (BM-MSC/AT-MSC n=7) and placentas (PT-MSC n=6). Passage 2 MSC were maintained in culture or cryopreserved, and viability, proliferation, migration, differentiation (osteogenic, adipogenic, and chondrogenic lineages), and expression of bioactive factors (VEGF, TGF, and bFGF) were compared.

Results: A significant decrease in viability was observed in all MSC immediately after cryopreservation, with PT-MSC being less affected than AT-MSC and BM-MSC. Viability was recovered in all MSC 24 h post-cryopreservation. In general, cryopreservation did not affect proliferation, migration, differentiation, or expression of bioactive factors.

Conclusions: Bovine fetal MSC from different tissue sources do not respond equally to cryopreservation regarding viability, but this process does not affect their intrinsic properties.

Keywords: bovine fetal MSC, placental MSC, cryopreservation.

Funding: National Agency for Research and Development (ANID, Project FONDECYT 11180681).

Oral and Posters Sessions

Breastfeeding

Characterization of breastmilk lipoproteins and evaluation of proatherogenic functions in women with obesity and dyslipidemia

Gabriela Arenas[1], Claudette Cantin[1], Andrea Morales Garrido[1], Andrea Leiva[1], Susana Contreras[2]

(1) Universidad San Sebastian, Faculty of Medicine and Sciences, Santiago, Chile. (2) Universidad San Sebastian, Faculty of Health Care Sciences, Santiago, Chile

Background: Dyslipidemia (D) and obesity (Ob) can affect women during the postpartum period when lactation occurs. Breastmilk (BM) major components include lipids such as total cholesterol (TC) and triglycerides (TG), as well as proteins. The presence and function of lipoproteins (LP) in BM, and how BM can be modified in presence of Ob and D are unknown. Here we characterized BM-LP and evaluated their proatherogenic function in women with Ob and D. Methods: Clinical characteristics of the recruited women were analyzed (ethics approval #492021-20, Universidad San Sebastian). Women were categorized as normal weight (N), Ob, and Ob-D, and their BM were analyzed. BM-LP isolation was performed by ultracentrifugation, and density was determined. The presence of apolipoprotein-B (apo-B) in BM-LP was determined by western blot. TC and TG were determined enzymatically in whole-BM and BM-LP. Proteins were measured by MicroBCA. Conjugated dienes and reactive oxygen species (ROS) were performed to determine pro-oxidant capacity from BM-whey and BM-LP, respectively. Thiobarbituric acid reactive substances (TBARS, malondialdehyde (MDA) levels) were performed to evaluate lipid peroxidation in whole-BM. Results: Clinical parameters were similar between groups excepting weight, BMI, and lipids, that were increased in Ob and Ob-D, respect to N. BM-LP-like were identified, in a range of density among VLDL, IDL, and LDL. BM-LP-like were confirmed with ApoB, in the first months of lactation. ApoB abundance was increased in Ob-D women respect to N and Ob in BM-LP-like and whole-BM, together with total proteins. TC and TG did not present changes in whole-BM and BM-LP-like. MDA-levels from BM-LP-like were increased in Ob-D women, compared with Ob and N. Conjugated dienes, and ROS did not present changes between groups. Conclusions: LP-like are present in BM during the first months of lactation. These BM-LP-like showed high MDA and proteins levels in Ob-D group compared with N, and Ob. Keywords: breastmilk-lipoproteins, obesity, dyslipidemia

Financing: Subvención a la instalación en la academia SA77210098, FONDECYT Regular 1230527.

Association of commensal bacteria and immunoglobulins with regulatory T lymphocytes in human colostrum

Mextli Y. Bermejo Haro[1,2], G. Hernández-Peláez[1], D.S. De la Merced-García[1], J.A. Mendoza-Ortega[2,3], C. Domínguez-Vanegas[1], R. T. Camacho-Pacheco[1,2], Y. Brito-Pérez[1], R. Figueroa-Damián[1], S. Rodríguez-Martínez[2], I. Mancilla-Herrera[1], M.A. Nájera-Hernández[1,2]

(1) National Institute of Perinatology (INPer) Infectology and Immunology Dept. (2) National Polytechnic Institute, Immunology department, National School of Biological Sciences (3) INPer, Dept. of Bioinformatics and Statistical Analysis. Ciudad de México, Mexico

Breast milk is the best source of nutritional factors, bacterial and defending elements which are required for breastfed infants in the first years of life. Colostrum is the richest state of milk containing commensal bacteria, antibodies, and lymphocytes. The diversity, affinity or phenotype of these components resemble those found in maternal enteric mucosa, and it has been suggested that are transported by a maternal entero-mammary pathway. In the gut mucosa, commensal bacteria, IgA, and Treg are interrelated to maintain host tolerance and defense, so in this work, we show a descriptive analysis of the correlation between the most abundant commensal bacteria, immunoglobulin isotypes, and Treg cells in human colostrum. This study was performed in accordance with the ethical standards laid down in the Declaration of Helsinki and approved by the Research, Biosecurity and Ethics Committees at the National Institute of Perinatology (INPer), Mexico City. 33 samples were donated by healthy women; by flow cytometry memory Treg lymphocytes were identified (CD45++CD3+CD4+CD25++CD45RO+CD127-), being the 24% +/- 13.16 out of the total lymphocytes. Furthermore, immunoglobulins were quantified by immunoassays, IgA was the principal immunoglobulin in colostrum (2207.6 ng / mL), followed by IgG3 (1121.3 ng / mL), IgG1 (100.6 ng / mL), IgG4 (29.6 ng / mL), IgG2 (10.6 ng / mL) and IgM (2.6 ng / mL). In addition, DNA of commensal bacteria were quantified by qPCR; Streptococcus and Staphylococcus were the predominant isolated genus in colostrum (46.6% and 44.86% respectively), followed by Bifidobacterium (3.76%), Enterococcus (3.4%) and Lactobacillus (1.37%). Correlation and multivariate analysis determinate that the presence of Treg was positively associated with the concentration of Bifidobacterium, and both negatively does with the concentration of IgA. This study shows for the first time, in human colostrum, a Lymphocyte Treg-IgA-Bacteria axis which could help to maintain the newborn homeostasis.

Keywords: Treg, Commensal bacteria, Bifidobacterium, IgA, Breastmilk, Colostrum

Financing: Instituto Nacional de Perinatología (INPer) Isidro Espinosa de los Reyes; Registration: 3120-20706-01-16. Acknowledgments: Postgraduate degree in immunology from ENCB-IPN. Department of Immunology and Infecto

Pregestational obesity moderates the protective effect of 6 months of exclusive breastfeeding against infant obesity at 2 years.

Karina Carrasco[1,2], José A. Castro-Rodríguez[3], Paola Casanello[1,4]

(1) Pontificia Universidad Católica de Chile, Santiago, Chile, Department of Obstetrics, School of Medicine, Santiago, Chile. (2) Universidad de Chile, Public Health PhD Program, School of Public Health., Faculty of Medicine, Santiago, Chile. (3) Pontificia Universidad Católica de Chile., Department of Respiratory Pediatrics., School of Medicine, Santiago, Chile. (4) Pontificia Universidad Católica de Chile, Department of Neonatology,, School of Medicine, Santiago, Chile

Background. Pregestational obesity (PGO) is a major public health problem and increases the risk of obesity in the offspring. Exclusive breastfeeding (EBF) could be a protective factor against infant obesity. However, it is not clear if PGO could have a modifying role in the relationship between EBF and infant obesity. The aim of this study was to analyze the relationship between 6 months of EBF (EBF-6m) and infant nutritional status at two years, evaluating the role of maternal PGO.

Methods. In a prospective cohort study from birth to 2 years (NCT02903134), 328 healthy women with singleton pregnancies delivered at Sótero del Río Hospital in Santiago, were enrolled. Pregestational body mass index was calculated (<14 weeks of gestation). At 6 months postpartum, a breastfeeding report was requested. At 2 years, infants with EBF-6m (n=48) and those without (n=61) were compared. The relationship between EBF-6m and the infant`s weight, BMI, weight-age z-score and BMI-age z-score were examined by bivariate analysis. The role of PGO in these infant outcomes was evaluated through one-way ANOVA and multivariate linear regression models.

Results. At 2 years, there were no differences in the nutritional status between infants with EBF-6m and those without. However, the infants from mothers with PGO, without EBF-6m had 1.10 kg (p: 0,0022), 0.9 BMI score (p: 0,0011), 0.8 weight-age z-score (p: 0.0031) and 0.65 BMI-age z-score (p: 0.0018) higher than those from mothers with normal weight and EBF-6m. Nutritional status was similar between the infants from mothers with normal weight and PGO and with EBF-6m. In linear regression analysis, adjusting by the sex of the child, the same results were obtained.

Conclusions. In this group of infants, 6 months of EBF had a protective effect against early markers of infant obesity at 2 years.

Keywords: Maternal obesity; postnatal growth; exclusive breastfeeding

Financing: FONDECYT 1141195 (JAC) & 1221812 (PC) and ANID PhD scholarship 21210843 (KCN)

Persistent organic pollutants in human milk and the Brazilian's infant development throughout the first year

Ana Lorena Ferreira[1], Nathalia Freitas-Costa[1], Samary Freire[1], Amanda Cunha Figueiredo[1], Marina Padilha[1], Nadya Helena Alves-Santos[2], Gilberto Kac[1]

(1) Rio de Janeiro Federal University, Nutritional Epidemiology Observatory, Rio de Janeiro, Brazil. (2) Federal University of South and Southeast of Pará, Institute for Health and Biological Studies, Faculty of Collective Health, Marabá, Brazil

Background: Persistent organic pollutants (POPs) are ubiquitous compounds that bioaccumulate in human like human milk (HM)1. Exposure to POPs is associated with infant development (ID). Although positive aspects of breastfeeding seem to overcome the risk of unfavorable outcomes from POPs exposure3, it is vital to understand the subclinical impact that pollutants in HM may have on early childhood development. We aimed to explore the association between POPs concentration in HM at 2-50 days postpartum and ID throughout the first year postpartum. Methods: A cohort of 68 healthy adult Brazilian women and their infants were followed from 28-35 gestational weeks to 12 months postpartum. HM samples were collected between 2-50 days postpartum, and POPs concentrations were analyzed using gas chromatography with mass spectrometry. ID was evaluated according to Age and Stages Questionnaires at 1 (n=66), 6 (n=50), and 12 months (n=45). Linear mixed-effects models were used to investigate the association of POPs in HM with ID, adjusted by birth weight, gestational weight gain, gestational age at birth, usual energy intake during pregnancy, maternal schooling and age, pre-pregnancy BMI and parity. Benjamini-Hochberg correction for multiple testing was performed. P< 0.1 was considered for interaction models with time. The study was approved by the Research Ethics Committee. Results: Forty-seven percent of the participants presented pre-pregnancy overweight/obesity, 58% self-reported to be brown/mixed color, 56% were primiparous, and 82% of the infants presented adequate birth weight for gestational age. The adjusted interaction models showed inverse associations between total POPs (β=-0.000002, P=0.10), total organochlorine pesticides (β=-0.000002, P=0.10), and dichlorodiphenyldichloroethylene concentrations in milk (β=-0.000002, P=0.10) and fine motor skills scores. Conclusions: Our exploratory findings revealed that postnatal exposure to organochlorine pesticides in HM could negatively impact the trajectories of fine motor skills of infants from R. Janeiro, Brazil.

Keywords: Breast milk, early childhood development, organochlorine pesticides

Financing: Carlos Chagas Filho Foundation for Research Support of Rio de Janeiro State-FAPERJ (E-26/210.190/2014, E-26/010.002429/2019, and E-26/200.429/2020). National Council for Scientific and Technological Development-CNPq (409676/2016). Acknowledgments: We gratefully acknowledge all cohort women and infants from the Public Health Center, where the data were collected.

Analysis of two interventions and breastfeeding duration

Francisco Mardones[1], Álvaro Erazo[1], Raúl Caulier-Cisterna[2], Patricia Zamora[3], Isabel Pereyra[4]

(1) Latin American Center for Economic and Social Studies (CLAPES) and (2) Center for Biomedical Imaging, Radiology department, Pontificia Universidad Católica de Chile, Santiago, Chile, (3) Ministry of Health, Department of Nutrition and Food (4) Universidad Católica del Maule, School of Nutrition, Health Sciences, Curicó, Chile

Background: Exclusive breastfeeding (EBF) practice has decreased in Chile since the first decades of the last century (1). Factors considered as determinants of that historical trend have been: a) healthcare teams' practices that discourage breastfeeding and b) a progressive increase of women working full-time outside their homes (World Rev Nutr Diet. 1989; 58,1-32; Arch Latinoam Nutr. 2004; 54, 374-379; Rev Chil Pediatr. 1988; 59, 347-348). The Government implemented in 1996 a long-running Breastfeeding Promotion Program (BPP) aimed to improve public health personnel's knowledge and skills. The Government also passed a law in 2011 that increased maternity leave postpartum from 12 to 24 weeks for fully-employed women.

The possible impact on the EBF duration of the BPP and the Maternity Leave Expansion (MLE) was studied.

Methods: An ecological design analyzed grouped data from 1993 until 2018 at three and six months of life with representative samples of the infant population cared for by the Ministry of Health outpatient clinics with data published before and after each intervention. Statistical methods included directly standardizing EBF rates and calculating the rates ratio (RR) and the etiological fraction (EF). Ethics: this study just used data from published reports.

Results: Samples showed trends of increasing EBF standardized rates at the third and the sixth months (p-values were < 0.01, applying the Chi-squared test and Bonferroni as sensitivity analysis) in association with the two national interventions. The relative changes in the RR allowed to estimate that the MLE national intervention may be more effective in improving EBF frequency. The higher EF values at 3 and 6 months were also observed in the MLE period beginning in 2011.

Conclusions: The comparison of two national public interventions using an ecological design resulted in similarly strong associations with EBF duration, including an apparent higher impact of the MLE.

Financing: This research received no specific grant. Acknowledgments: We recognize the hard work of the health pesonnel who gathered all the information for this studiy at the health clinics.

Increased PPARα activity during lactation prevents the development of obesity and metabolic dysfunction programmed early in life.

Lucas P. Jacinto Saavedra[1], Scarlett Rodrigues Raposo[1], Naiara C. Lucredi[2], Ana L. Manso Assakawa[1], Maria N. Chimirri Peres[1], Veridiana M. Moreira[1], Letícia F. Barbosa[1], Diana Sousa[3], Silvano Piovan[6], Gessica D. Gonçalves[1], Flávia C. Farias dos Santos[4], Jones Bernardes Graceli[4], Jurandir Fernando Comar[2], Rodrigo Mello Gomes[5], Paulo Matafome[3], Douglas Lopes Almeida[1], Paulo Cezar de Freitas Mathias[1]

(1) St. University of Maringá, Dept. of Genetics Cell Biology and Biotechnology and (2) Dept. of Biochemistry, (3) Polytechnic Institute of Coimbra, (4) Federal University of Espírito Santo, Dept. of Physiology, (5) Federal University of Goiás, Dept. of Physiology. (6) Western Paraná St. University, Dept. of Biochemistry.

Background: Litter reduction (SL) leads to post-natal overfeeding, causing alterations that reassembles very well childhood obesity a condition of major concern. The activation of PPAR-alpha at adulthood, a major regulator of lipid metabolism, was shown to induce lipid oxidation and prevent diet-induced obesity and metabolic dysfunction. We hypothesize that increased post-natal PPAR-alpha activation can prevent obesity and metabolic dysfunction induced early in life. Methods: On post-natal day 3 (PN3), male Wistar rat offspring had their litter reduced (3 pups per dam, SL, n=8), and the control litter (9 pups per dam, NL, n=9). Animals were evaluated at adulthood (PN120) for metabolic and biometrical markers. To verify if increasing PPAR-alpha during perinatal period would prevent the phenotype induced by SL, SL offspring, were treated during lactation (PN1 to PN21), with an agonist of PPAR-alpha, fenofibrate (12.5mg/kg, SL-F, n=11), or vehicle (SL-V, n=9) and were evaluated at adulthood (PN120). The animals had ad libtum access to food and water. Approved by the Ethic Commission in The Use of Animals (CEUA) from State University of Maringá, Brazil (Protocol Number 8934110422). Results: SL, leads to visceral obesity ($p<0.05$), insulin resistance (Kitt, $p<0.05$), increased hepatic triglycerides (TGs) ($p<0.05$), microsteatosis ($p<0.05$) and, increased hepatic FGF21 expression ($p<0.05$) and decreased sympathetic nerve activity of brown adipose tissue (BAT) ($p<0.01$), also decreased expression of the receptor for FGF21, FGFR1, in the hypothalamus ($p<0.01$). Increased postnatal PPAR-alpha activity prevents at adulthood visceral obesity ($p<0.05$), insulin resistance ($p<0.05$), hepatics TGs accumulation ($p<0.05$) and microsteatosis ($p<0.05$), decreased autonomic sympathetic signaling of BAT ($p<0.05$), and decreased expression of FGFR1 in the hypothalamus ($p<0.05$). Conclusions: Increased post-natal PPAR-alpha activity during lactation prevents visceral obesity, insulin resistance and BAT autonomic sympathetic hypoactivity. Increasing PPAR-alpha activation during lactation could be a useful intervention to prevent the development of obesity and its comorbidities during adult life. Keywords: childhood obesity, small litter, ppar-alpha. Financing: JBS “Fazer o bem faz bem”; CAPES; National Council for Scientific and Technological Development (CNPq).

Anti-SARS-CoV-2 IgA and total antibodies levels in breast milk of infected women three months postpartum.

Letícia Veríssimo Dutra[1], Mariliza Henrique Silva[3], Cibele Wolf Lebrão[3], Fernando Luis Affonso-Fonseca[2], **Fabíola Isabel Suano-Souza[1,2]**

(1) Federal University of Sao Paulo, Pediatrics, São Paulo, Brazil. (2) University Center Faculty of Medicine of ABC, Pediatrics, Santo André, Brazil. (3) University Municipal Hospital of São Bernardo do Campo, São Bernardo do Campo, Brazil

Background. Studies describe the presence of anti-SARS-CoV–2 IgA and neutralizing antibodies in the breast milk (BM) of women who had COVID-19 infection during pregnancy and lactation1. There are few studies evaluating the durability of anti-SARS-CoV-2 antibodies in BM, some studies have identified their presence for 4 to 10 months after infection, however, the results of maternal COVID-19 during pregnancy and childbirth in the newborn (NB) in the long term are still insufficient. To evaluate the presence of anti-SARS-CoV–2 IgA, IgG and total antibodies (TA) in BM of women who had COVID-19 infection during pregnancy, childbirth or the puerperium, with 3 months of lactation. Methods. This study was approved by the Research Ethics Committee of HMU-SBC and FMABC (CAAE: 34587520.3.0000.0082, opinion4.184.253).A prospective cohort study was carried out in the HMU-SBC with pregnant and parturient women who had COVID-19 infection and their newborn. A total of 261 pairs were included at birth and, of these, 85 attended the follow-up at 3 months postpartum. Data collected: weight, length and head circumference of the NB at 3 months of age, complications presented by the infant and maternal weight and height. Blood samples (5 mL) were collected from the woman and the infant, in addition to 5-10 mL of BM at 3 months of lactation to measure IgA, IgG and total antibody (TA). Results. At 90 days of age, 61(71.8%) of the infants were breastfed and all had adequate anthropometric indices.The initial descriptive analysis showed the presence of anti-Sars-CoV-2 IgA and TA(neutralizing) in 65.3% and 72.2%, respectively, of the evaluated BM samples. This positivity dropped significantly after 90 days, reaching 14.1% and 37.1%, respectively. Conclusions. Anti-SARS-CoV-2 antibodies in BM seem to decrease at 3 months postpartum, however, their presence confers protection to the breastfed infant. These findings reinforce the importance of breastfeeding and maternal passive immunity for the baby.

Keywords: Coronavirus Infections, Pregnancy, Newborn, Breastfeeding

Financing: CAPES and FAPESP 2021/04784-2. Acknowledgments: CAPESFAPESPCNPQUniversity Center Faculty of Medicine of ABCUniversity Municipal Hospital of São Bernardo do CampoFederal University of Sao Paulo

Breastfeeding practices and trajectories of BMI-for-age in infants from the Brazilian primary health care system

Nathália Teixeira de Oliveira[1], Dayana Rodrigues Farias[1], Gilberto Kac[1], Raquel Machado Schincaglia[1], Keronlainy Silva Salvatte[1], José Thiago Alves de Sousa[1], Isabela Rodrigues dos Santos Silva[1], Amanda Melo de Albuquerque[1], Thais Rangel Bousquet Carrilho[1]

(1) Rio de Janeiro Federal University, Josué de Castro Nutrition Institute, Nutrition, Av. Carlos Chagas Filho,373 - Bloco J2 - sala 29 - Cidade Universitária - Rio de Janeiro, Rio de Janeiro, Brazil

Background: The weight gain velocity early in life is correlated with body composition and the long-term risk of obesity. However, there is limited knowledge regarding the role of breastfeeding practices (BFP) on body mass index (BMI) trajectories. Thus, this study aimed to evaluate the association between those practices between 4-5 months and the trajectories of BMI for age (BMI/A) from 6 to 23 months. Methods: This longitudinal study used data from 131,222 children registered in the Brazilian National Food and Nutrition Surveillance System between 2015 and 2019. Data on food consumption between 4-5 months and at least one measure of weight and length between 6-23 months were used. Food consumption data were based on a structured questionary on food consumption markers. BMI/A was evaluated in z scores according to the WHO standards. Results were presented as prevalence and 95% confidence intervals (95% CI). Linear mixed-effects models were used to estimate the association between BFP and BMI/A, including the interaction between BFP and child age, and adjusted for sex, participation in a conditional cash transfer program, and macro-regions. Ethics Committee:(CAAE: 18447919.3.0000.5264, Opinion: 3.528.976, approved on August 23, 2019). Results: Most children were not in EBF at 4-5 months (66.40%, 95%CI= 66.27;66.53). It was observed a 2.1% prevalence of underweight (95%CI= 1.89;2.36) and 33.2% of overweight (95%CI=32.42;33.95) at 22-23 months. A statistically significant effect of the interaction between BFP and the child's age on BMI/A was observed. Children classified in mixed breastfeeding (βinteractionBFP#age=0.19; 95%CI= 0.17; 0.22; p<0.001), complementary (βinteractionBFP#age=0.09; 95%CI= 0.07; 0.11; p<0.001) and no breastfeeding (βinteractionBFP#age=0.21; 95%CI= 0.19; 0.24; p<0.001) had higher velocity in BMI/A than children on EBF. Conclusions: Children who were not exclusively breastfed, especially those not breastfed, at 4-5 of age had higher velocity in BMI/A trajectory between 6-23 months.

Keywords: Infant, Nutrition Status, Breastfeeding

Financing: Research Support Foundation of the State of Rio de Janeiro (FAPERJ)

Evaluation of health professionals' guidelines on breastfeeding: duration of the practice and mothers' satisfaction.

Andressa Vivas[1], Fabíola Isabel Suano de Souza[2]

(1) Universidade Federal de São Paulo, Department of Pediatrics, Escola Paulista de Medicina, Rua Botucatu, 740, São Paulo, São Paulo
(2) Universidade Federal de São Paulo, Department of Pediatrics, Escola Paulista de Medicina, Rua Botucatu, 740, São Paulo, São Paulo

Background: In the last 40 years, public policies were created in Brazil to encourage breastfeeding. As a result, the percentage of exclusive breastfeeding until 6 months increased and nowadays reaches 45,8%1. Despite the progress, this prevalence is far from the World Health Organization's (WHO) goal to 2030, according to which 70% of the babies should receive exclusive breastfeeding until 6 months.
Methodology: This cross-sectional study included primiparous mothers of babies between 6 and 24 months of age and was approved by the ethics committee.The mothers answered a questionnaire that assess the quantity and the quality of the informations about exclusive breastfeeding provided by health professionals, as well as the duration of exclusive breastfeeding and the mother's satisfaction with breastfeeding.
Results until now: There are 44 women included in the research: 39 (88.64%) live in São Paulo State, the majority (35 - 79.54%) are college graduate or postgraduate, and the minority (6 - 13.64%) knows about The International Code of Marketing of Breast-milk Substitutes. The results imply the importance of maternity leave to the success of breastfeeding: 70.96% of the working mothers were contemplated with maternity leave and 75% of the participants breastfed exclusively for up to 6 months. There was a high rate of C-section among the participants (28 - 63.64%), although 23 (52.27%) delivered in a Baby-friendly Hospital. Furthermore, the mothers have conflicting feelings about breastfeeding, because, despite the fact that they describe the breastfeeding as physically (61.36%) and emotionally (43.18%) exhaustive, 90.9% of them refer positive feelings about breastfeeding. Finally, much of the informations about breastfeeding not provided to the mothers are related to their body and health.
Conclusions: It is noticed that women receive informations from health professionals about breastfeeding; otherwise, the informations about the care for physical and mental health of mothers are still insufficient.

Keywords: breastfeeding, mother's satisfaction, health professionals

Financing: The author receives a scholarship (Scientific Initiation) from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo).

Cancer

Effect of cannabidiol and the potential role of TRPV2 on the viability of PC3 cells

Kevin Brockway GV[1,2], María José Torres Torres[1], Sebastián Indo[2,3], Enrique A. Castellón[2,4], Héctor R. Contreras[2,4]

(1) Pontificia Universidad Católica de Chile, School of Chemistry and Pharmacy, Faculty of Chemistry and Pharmacy, Av. Vicuña Mackenna 4860, Santiago, Chile. (2) Universidad de Chile, Department of Basic-Clinical Oncology, Faculty of Medicine, Av. Independencia 1027, Santiago, Chile. (3) Universidad de Chile, Department of Medical Technology, Faculty of Medicine, Av. Independencia 1027, Santiago, Chile. (4) Center for Cancer Prevention and Control (CECAN), Independencia 1027, Santiago, Chile

Background. Cannabidiol (CBD) is one of the main constituents of the Cannabis sativa plant and interacts with specific CB1 and CB2 receptors, and with TRPV channels. Various studies have shown that TRPV2 is highly expressed in metastatic prostate cancer cell (PC) lines. Previous work in our laboratory demonstrated that treatment with cannabinoids increases the percentage of apoptotic cells. Therefore, the objective of this work was to study the effect of CBD and the potential role of the TRPV2 receptor on the viability in PC3 cells.

Methods. The expression and localization of TRPV2 in RWPE-1 cells (prostatic epithelium cells), PC3 (CP cells from bone metastases) and DU145 (CP cells from brain metastases) were determined by western blot, RT-qPCR and immunofluorescence, respectively. In parallel, the functionality of the channel in PC3 cells through the autoregulation of TRPV2 expression was determined by western blot. Next, the effect of CBD on viability in PC3 cells using a selective TRPV2 inhibitor was evaluated by MTT assays. The results were expressed as the mean + standard deviation of three individual experiments (Kruskal-Wallis or Tukey multiple comparisons Test).

Results. Our results indicate higher levels of TRPV2 mRNA and protein in PC3 cells compared to RWPE-1 and DU145 cell lines, and channel localization is preferentially distributed in the plasma membrane. On the other hand, PC3 cells stimulated with CBD increased the expression of TRPV2. Additionally, CBD and TRPV2 blockade exert a cytotoxic effect showing a reduction in viability in PC3 cells.

Conclusions. These findings show that cell lines representing advanced stages of PC express high levels of TRPV2, and that CBD and TRPV2 blocking have a cytotoxic effect, suggesting that cannabinoids could be a beneficial option for PC treatment.

Keywords: Cannabidiol (CBD), TRPV2, Viability, Prostate Cancer.

Financing: Fondecyt Postdoctoral Project 3210213 (MJT), Fondecyt Regular Project 1201704 (EC and HC). ANID, FONDAP 152220002.

Effect of metformin and NSAIDs in cell viability of EOC cell lines treated with cisplatin

Maritza P. Garrido[1,2], Renzo Adriazén[1], Omar Painen[1], Lucas Miralles[1], Allison Fredes[1], Carmen Romero[1,2]

(1) Laboratory of Endocrinology and Reproductive Biology, Clinical Hospital University of Chile. (2) Obstetrics and Gynecology Department, Faculty of Medicine, University of Chile

Background: Epithelial ovarian cancer (EOC) is a lethal gynecological malignancy. Current treatment in advanced stages of EOC is cytoreductive surgery and platinum-based chemotherapy. Unfortunately, resistance to cisplatin is usual, being necessary to study new therapy approaches. In this line, well-known drugs such as metformin or non-steroidal anti-inflammatories (NSAIDs) have shown anti-tumoral properties in retrospective cohorts and preclinical models.

Aim: To study the effect of therapeutical doses of metformin and/or NSAIDs on the cell viability of EOC cells treated with cisplatin.

Methods: EOC cell lines A2780, OV90, and A2780 cisplatin-resistant (A2780-cis) were stimulated with different concentrations of metformin, diclofenac, and indomethacin in the presence and absence of cisplatin. CCK-8 assays were performed in four different experiments to realize dose-response curves. The analysis of results was done using three-parameters logistic regression and the Mann-Whitney test. A $p<0.05$ was considered significant.

Results: The inhibitory concentration 50 (IC50) of indomethacin was lower in A2780-cis compared to A2780 or OV90 cells (143 vs 311 and 235 μM respectively), while the IC50 of diclofenac was 30, 46, and 75 μM for A2780, Ov90, and A2780-cis respectively. The combined use of metformin (20 μM) plus diclofenac (5 μM) increases the response of A2780 and A2780-cis to cisplatin treatment, compared to cisplatin alone ($p<0.05$).

Conclusions: Results indicate that diclofenac and indomethacin have cytotoxic effects in EOC cells. Additionally, the use of repurposing drugs (drugs used for new purposes) such as metformin and diclofenac, in low concentrations, could sensibilize EOC cells to cisplatin. Therefore, the use of these drugs as complementary therapy could be beneficial against EOC. Ethical issues: This study was approved by the local Scientific Ethics Committee (Record 34, 2023).

Keywords: Ovarian cancer, metformin, NSAIDs

Financing: Funding: FONDECYT regular 1220479 (CR) and Concurso semilla 2022 Hospital Clínico Universidad de Chile (1349/23) (MG)

Rest overexpression decreases migration, invasion and viability to enzalutamide in 22rv1 cells

Sebastián Indo Cofré[1,4], Octavio Orellana-Serradell[2], María José Torres Torres[3], Enrique Castellón Vera[4,5], Héctor R. Contreras Muñoz[4,5]

(1) University of Chile, Department of Medical Technology, Faculty of Medicine, Independencia 1027, Santiago, Chile. (2) University of Chile, Millennium Nucleus of Ion Channel-Associated Diseases (MiNICAD), Faculty of Medicine, Santiago, Chile. (3) Pontificia Universidad Católica de Chile, Laboratory of Molecular Pharmacology and Toxicology, Faculty of Chemistry and Pharmacy, Santiago, Chile. (4) University of Chile, Laboratory of Oncology Cellular and Molecular, Department of Basic-Clinical Oncology, Faculty of Medicine, Santiago, Chile. (5) Center for Cancer Prevention and Control, Santiago, Chile

Background. REST is a repressor factor of the neural phenotype, which is decreased in neuroendocrine prostate cancer (NEPC). NEPC represents a poor prognostic stage, which emerges from castration-resistant prostate cancer (CRPC). The appearance of NEPC has been associated with the phenomenon of transdifferentiation, possibly stimulated by the epithelial-mesenchymal transition (EMT) process in cells that have been subjected to androgen deprivation therapy (ADT) and enzalutamide. The objective of this work is to determine if the overexpression of REST in the 22rv1 cell line (xenograft-derived prostate cancer) represses the EMT process, migration, and invasion, in addition to evaluating the viability to enzalutamide. Methods. The expression of EMT regulatory genes, such as Twist and Zeb1, and the androgen receptor (AR) were evaluated through RT-qPCR, and western blot in nuclear and cytosolic fractions of 22rv1 cells with REST overexpression (22rv1-REST). The migratory and invasive capacities of the overexpressing cells were evaluated by Transwell® assays with and without Matrigel, respectively, and the viability to enzalutamide was determined with MTT assays. Results. 22rv1-REST cells showed decreased protein nuclear level of Twist, Zeb1, and AR, together with decreased migration and invasion. The 22rv1-REST cells presented lower viability to enzalutamide compared to the control line. Results were expressed as the mean + standard deviation of at least three independent experiments (Mann Whitney U test, Kruskal-Wallis, or Tukey test for multiple comparisons). Conclusions. Our results support the fact that, in our model, REST behaves like a tumor suppressor, decreasing the aggressive behavior of 22rv1 cells, probably through the repression of the EMT process and neuroendocrine phenotype. Furthermore, REST could represent a marker of response to enzalutamide in patients with prostate cancer.

Keywords: Prostate cancer, REST, epithelial-mesenchymal transition, NEPC

Financing: U-Apoya (Links) 22-2019, regular FONDECYT #1201704 and ANID-FONDAP #152220002.

Characterization of primary cell cultures as study models for advanced prostatic cancer.

Camila Leal[1,2], Hector Contreras[1,2], Enrique Castellon[1,2]

(1) University of Chile, Department of Basic and Clinical Oncology, Faculty of Medicine, Independencia 1027, Santiago, Chile. (2) Center for Cancer Prevention and Control (CECAN)

Background: Metastatic and castration-resistant prostate cancer (mCRPC) is a significant therapeutic challenge. Approximately 10-20% of patients develop castration resistance, leading to mCRPC. Epithelial-to-mesenchymal transition (EMT) in cancer progression is a complex process in which a transformed epithelial cell gives rise to a mesenchymal-like malignant cell. In this process, a diversity of tumor cells can be produced, including cancer stem cells (CSCs), which are implicated in metastasis, recurrence, and resistance. Transcription factors such as SOX2 are associated with EMT and CSC pluripotency. SOX2 overexpression has been linked to resistance to anti-androgen therapies and increased EMT/CSC expression. Methods: Prostate cancer (PCa) samples from two patients were used. Two primary cell cultures were obtained (cPCa-1 and cPCa-2) and characterized by qPCR and western blot techniques, to analyze gene and protein expression and quantification of different markers, as SOX2, KLF4, and ZEB1. Data were analyzed by a Mann-Whitney t-test (n=4) and $p<0.05$ was considered significant. Protocols for tissue collection, use and processing have been approved by the institutional Ethical Committee of Faculty of Medicine, University of Chile n° 125-2015 and 083-2020. Results: According to the cell morphology, EMT, and stemness markers, cPCa-1 culture presented characteristics of advanced cancer showing high expression of SOX2, KLF4, and ZEB1, transcription factors that can be related with a mesenchymal phenotype, which would be correlated with a more aggressive tumor. Instead, cPCa-2 cells showed lower expression of those markers providing a less agressive model. Conclusions: Primary PCa cell cultures with high expression of stemness and EMT markers provide a suitable model for the study of aggressiveness features such as recurrence and resistance to therapies. This model could represent a better approach to PCa progression. Understanding the role of SOX2 and other transcription factors in chemoresistance and its association with a mesenchymal and stem cell phenotype could provide insights into PCa aggressiveness.

Keywords: SOX2, castration-resistant prostate cancer, epithelial-to-mesenchymal transition.

Financing: Funding: Fondecyt 1201704 and ANID, FONDAP 152220002

Effect of alpha-lipoic acid-mediated decrease of LTB4 production on the viability, proliferation, and migration of non-small lung cancer cells

María José Torres Torres[1], Alexandra Valle[1], Kevin Brockway GV[1,2], Sebastián Indo[2,3,] Enrique A. Castellón[2,4], Héctor R. Contreras[2,4], Mario Faúndez[1]

(1) Pontificia Universidad Católica de Chile, School of Chemistry and Pharmacy, Faculty of Chemistry and Pharmacy, Avenida Vicuña Mackenna 4860, Santiago, Chile. (2) Universidad de Chile, Department of Basic-Clinical Oncology, Faculty of Medicine, Santiago, Chile. (3) Universidad de Chile, Department of Medical Technology, Faculty of Medicine, Santiago, Chile. (4) Center for cancer prevention and control (CECAN), Santiago, Chile

Background. Leukotriene B4 (LTB4) is a lipid mediator related to inflammatory processes whose production is catalyzed by the enzyme leukotriene A4 hydrolase (LTA4H). Previous studies have shown that in patients with non-small cell lung cancer, as the disease progresses, LTB4 levels increase. Currently, there are no drugs that specifically regulate the imbalance in the synthesis of LTB4. In the same sense, our research group demonstrated that alpha lipoic acid (ALA) inhibits recombinant human LTA4H, reducing LTB4 levels. Therefore, the objective of this work was to study the effect of ALA-mediated decrease in LTB4 production on the viability, proliferation, and migration of non-small lung cancer cells.

Methods. Interactions between ALA and the LTA4H catalytic site were evaluated through docking and molecular dynamics. In parallel, the expression of cPLA2, 5-LOX, FLAP, LTA4H, BLT1, and LTB4 production was determined in A549 cells (human adenocarcinoma alveolar basal epithelial cells) by western blot, RT-qPCR, and ELISA, respectively. Next, viability, proliferation, and migration in the same cell line were evaluated by MTT, western blot, and wound closure, respectively.

Results. Our results indicate that ALA interacts with essential amino acids for LTA4H activity. Additionally, A549 cells express cPLA2, 5-LOX, FLAP, LTA4H, BLT1, and produce LTB4, which are reduced by treating cells with ALA. Finally, ALA reduces viability and migration, with no change in proliferation in A549 cells. The results were expressed as the mean + standard deviation n>3 (Krustal-Wallis or Tukey multiple comparisons Test).

Conclusions. These findings suggest that LTB4 production by non-small lung cancer cells may promote disease progression, so ALA could be a beneficial option for lung cancer treatment.

Keywords: Leukotriene B4 (LTB4), alpha lipoic acid (ALA), non-small lung cancer cells, malignant phenotype

Financing: Fondecyt Postdoctoral Project 3210213 (MJT), Fondecyt Regular Project 1201704 (EC and HC)

DOHAD

Implications of gestational hypothyroxinemia over the maternal-fetal interface and the offspring's sex-dependent autistic-like outcomes.

Enrique González-Madrid[1,2], María A. Rangel-Ramírez[1,2], Oscar Álvarez[1,2], M. José Mendoza-León[1,2], Esteban Caamaño[1,2], Nicole Avilés Rivera[1,2], Alejandro Regaldiz[1,2], Gonzalo I. Cancino[3], Daniela Elgueta[3], María C. Opazo[2,4], Claudia A. Riedel[1,2]

(1) Universidad Andrés Bello, Facultad Cs. de la Vida. (2) Instituto Milenio de Inmunología e Inmunoterapia (IMII) y (3) Facultad de Cs. Biológicas, Pontificia Universidad Católica de Chile, (4) U. de las Américas, Facultad de Medicina Veterinaria y Agronomía. Stgo, Chile.

Background. Retrospective studies in humans have revealed an association between gestational hypothyroxinemia (HTX) and autistic traits in the offspring (1). Inflammation at the maternal-fetal interface also increases the offspring's predisposition to develop autism (2). Knowing that thyroid dysfunction triggers inflammatory responses (3), we aimed to investigate whether gestational HTX induces inflammation at the maternal-fetal interface and whether the adult HTX-gestated offspring manifests autistic-like outcomes. Methods. Gestational HTX was induced in pregnant mice (C57BL/6) by adding methimazole in the tap water during embryonic days (E)10 to E14. Control pregnant dams did not receive MMI (n=10). Mice were euthanized on E14. Placenta, uterus, decidua, and fetal brain were isolated, and cytokines were quantified in these tissues, as well as immune cell populations by ELISA and flow cytometry, respectively. Neuronal populations were assessed in fetal brains by immunofluorescence. Autistic-like behavior was analyzed in the offspring on P55, as well as cytokines and immune cell populations. Finally, the relative expression of NLGN3 and HOMER1 at the prefrontal cortex and hippocampus was evaluated by immunoblotting (n=15). Data were analyzed by two-way ANOVA and Tukey's post-test. Significance on $p<0.05$. Bioethics approval certificate number 012/2021 (Universidad Andrés Bello). Results. Pregnant mice induced with HTX had increased pro-inflammatory cytokines at the maternal-fetal interface and a higher proportion of granulocytic and inflammatory cells. HTX-gestated fetuses showed altered proportions of neuronal populations consistent with impaired neurodevelopment. The HTX-gestated offspring displayed an autistic-like behavior (both sexes) and inflammatory features including increased pro-inflammatory cytokines in serum, sex-dependent neuroinflammation traits, and reduced Treg/Th17 rate. Male HTX-gestated offspring showed elevated expression of NLGN3 and HOMER1 at the hippocampus and prefrontal cortex, as compared to controls. Conclusions. Gestational HTX generates an acute inflammatory state at the maternal-fetal interface, while the adult offspring display sex-dependent autistic-like outcomes. Financing: ANID DOCTORADO NACIONAL 21202085, 21211419, 21202356; Fondecyt 1191300, 1210507; Programa ICM-ANID, ICN2021_045, and UNAB DI-03-19/N. Beca de Asistencia Académica y Arancel, UNAB.

Early postnatal overfeeding combined with maternal under-nutrition modulates glycemia and endocrine islets area in normal body mass adult rats.

Douglas Lopes Almeida[1], Ananda Malta[1], LUCAS SAAVEDRA[1], Paulo Cezar de Freitas Mathias[1]

(1) Maringá State University, Genetics, Cell Biology and Biotechnology, Maringá, Brazil

Background. Previously our group showed that the combination of early life nutritional stresses may negatively impact adult glycemic homeostasis [1]. In this study, we tested the effects of the combination of maternal undernutrition with increased fed during lactation by postnatal litter reduction in the body mass, glycemic homeostasis and pancreatic endocrine islets area.

Methods. This work was approved by the local bioethics committee (CEUA nº 7952100423) and complied with all the Brazilian and International (ARRIVE) guidelines for animals' care and handling. Wistar rats' delivering was post-natal day 0 (PN0), the dams were divided in control diet (NP) and low-protein diet (LP) until PN14. On PN2 litters were adjusted to 9 (NL) or 3 (SL) pups, originating 4 groups: NP-NL, LP-NL, NP-SL and LP-SL. After PN14 to PN90, all animals received control diet. On PN90, animals from the 4 groups were subjected to an Intravenous Glucose Tolerance Test (IvGTT) or Insulin Tolerance Test (ITT), followed by euthanasia and tissue collection for ex vivo analysis.

Results. LP diet ($p<0.0001$) and litter reduction ($p<0.0001$) affected body mass in opposite ways, but LP-SL presented normalized body mass compared to control NP-NL. Albeit, the combination of early nutritional stresses lead to increased glucose intolerance ($p<0.001$) and higher peripheral insulin sensitivity (kITT $p<0.0001$) compared to the other groups, with a slight reduction in the pancreatic islet area ($p<0.05$).

Conclusions. The present results confirm our previous findings that shows accentuated decline in the glucose homeostasis of normal body mass adult male rats, with impairment of the endocrine pancreas development by later morphology differences. These plastic long-term effects in normal body mass adult male rats indicates that the complex environment of early life can affect mammal's key metabolism organs in different ways, depending on the type of the stress rather than body mass later in life.

Keywords: early-life nutrition, glucose homeostasis, body mass

Financing: National Council for Scientific and Technological Development – CNPQ – Grant number 164282/2020-3.

Consequences of gestational hypothyroxinemia on T regulatory lymphocytes in intestinal inflammation

Oscar Alvarez Mardones[1,2], Esteban Caamaño[1,2], Enrique Gonzalez Madrir[1,2], M. Jose Mendoza Leon[1,2], Maria A. Rangel Ramirez[1,2], Alejandro Regaldis[1,3], Felipe Melo Gonzalez[2,4], Maria C. Opazo[2,3], Susan M. Bueno[2], Pablo Gonzalez[2], Claudia Riedel Soria[1,2]

(1) Universidad Andrés Bello, Ciencias Biológicas, Ciencias de la Vida, Santiago, Chile. (2) Instituto Milenio en Inmunología e Inmunoterapia, Pontificia Universidad Católica de Chile/Universidad de Chile/ Universidad Andrés Bello, Santiago, Chile. (3) Universidad de las Américas, Santiago, Chile. (4) Laboratorio de inmunología Traslacional. Universidad Andrés Bello, Santiago, Chile

Background. Gestational hypothyroxinemia (HTX)is a frequent condition during pregnancy characterized by decreased plasma 3,5,3',5'-L-tetraiodo-tironina(T4) and normal 3,5,3'-L-tri-iodotironina(T3) and thyroid stimulating hormone(TSH) levels. Gestational HTX affects immune response in the offspring. We have shown by using animal models that gestational HTX increases the intensity of autoimmune diseases like experimental autoimmune encephalomyelitis (EAE) and ulcerative colitis (UC). In this study we analyzed the presence of T regulatory (Treg) and Th17 lymphocytes and the suppressor function of Treg lymphocytes in the offspring suffering UC. Methods. The Euthyroid-offspring and HTX-offspring were treated with 2% dextran sodium sulfate (DSS) for six days. The mice were euthanized, the spleen was obtained and Treg lymphocytes were in vitro activated with IL-2, anti-CD3 and anti-CD28 for seven days. After that, these cells were adoptively transferred into to euthyroid offspring, and 24 hour later UC was induced to receptor mice by 2% DSS in the drinking water for six days. The UC pathological score was registered each day and at day six mice were euthanized. The spleen was obtained and Treg and Th17 lymphocytes population were analyzed by flow cytometry. To evaluate Treg suppression capacity in vitro suppression assays were with TCD4+CD25+ lymphocytes co-cultured with T effector (Tefe) previously stained with CFSE and then proliferation of Tefe lymphocytes was analyzed by flow cytometry. The entire project has been approved by the bioethics committee of Andres Bello University. Results. Adoptive transfer experiments showed that those mice with UC that received Treg from HTX-offspring suffer strong UC symptomatology. The suppressor capacity of Treg in the HTX-offspring was reduced compared to control-offspring. Conclusions. The results from this work show that Treg suppressor capacity is reduced in the HTX-offspring and this can explain why the HTX-offspring suffers more intense autoimmune diseases like UC and EAE. Keywords. Gestational hypothyroxinemia, Treg lymphocyte, ulcerative colitis

Financing: Funding. Beca de Asistencia Académica y Arancel, UNAB. FONDECYT#1191300; #1180983. IMII P09/16-F. AAEC 2, 2023-2024.

Increased blood levels of zonulin in the adult progeny gestated under transient maternal hypothyroxinemia is associated with an autistic-like behavior

Nicole Avilés-Rivera[1,2], Enrique González-Madrid[1,2], Alejandro Regaldiz[1,2], Claudia A. Riedel[1,2]

(1) Laboratorio Endocrino-inmunología. Universidad Andrés Bello., Departamento de Ciencias Biológicas., Facultad de Ciencias de la Vida., Avenida República 330., Santiago., Chile. (2) Instituto Milenio Inmunología e inmunoterapia (IMII)., Santiago, Chile.

Background. Maternal thyroxine (T4) is essential for proper fetal neurodevelopment. Since the maternal thyroid gland must increase its function to cover maternal and fetal T4 demand, pregnant women are more likely to develop hypothyroxinemia (HTX), especially early in pregnancy. HTX is a condition defined by serum reduction of T4, with levels of T3 and TSH within the normal range. In humans, it has been reported that children are more likely to manifest autistic outcomes when the mother suffered HTX during the first gestational trimester. Autism spectrum disorder (ASD) is a neurodevelopmental condition, characterized primarily by alterations in communication and interaction and repetitive behaviors. An increase in blood zonulin, a protein that induces the opening of the tight junctions at the intestinal epithelium, has been observed in ASD patients. Moreover, adult mice gestated in HTX present autistic-like behaviors and alterations in intestinal permeability; however, the zonulin content in such animals is unknown. Methods. Pregnant mice (C57BL/6) were randomly distributed into two groups. To induce gestational HTX, the first group received methimazole in drinking water from E10 to E14. The second group did not receive such treatment. Female and male HTX and Control progeny (n=7-9 animals per group) in (P)55-62 were tested for repetitive and social behavior. In P63, the animals were euthanized and serum zonulin was quantified by sandwich ELISA. Statistical differences were evaluated using two-way ANOVA and considered significant when $p<0.05$. Bioethics approval certificate number 012/2021 (Facultad Ciencias de la Vida, Universidad Andrés Bello). Results. HTX progeny showed autistic behaviors compared to control progeny. Serum increases of zonulin was observed in both sexes of progeny gestated in HTX. Conclusions. These results suggest zonulin as a link between autistic behaviors and intestinal alterations in progeny gestated in HTX, and points to the need for monitoring thyroid status in early pregnancy.

Keywords: Gestational hypothyroxinemia, Autism-like outcomes, Zonulin

Financing: This work was funded by Fondecyt regular 1191300, Progama ICM-ANID, ICN2021_045 and the proyect N°DI-06-23/APP. Acknowledgments: PhD. Claudia Riedel Soria and PhD. student Enrique González-Madrid.

Ultrasonographic characterization of the Guinea Pig response to intrauterine chronic hypoxia.

FA Beñaldo[1], T Jimenez[1], N Lorca[1,2], J Mocarquer[1,2], AA Candia[3], AA Paz[1], A González-Candia[3], EA Herrera[1]

(1) Universidad de Chile, Vascular Function & Reactivity Lab, Pathophysiology Program, ICBM, Faculty of Medicine, Santiago, Chile. (2) Universidad de Chile, Resident of Maternal-Fetal Medicine, Faculty of Medicine, Santiago, Chile. (3) Universidad de O'Higgins, Institute of Health Sciences, Rancagua, Chile

Background. Small animal models, particularly guinea pigs (GP), have advanced knowledge of fetal disease programming1,2. However, the determination of physiological/pathological changes has been carried out at certain moments of pregnancy3, and there is no follow-up of these variables. Therefore, our objective was to perform the first non-invasive characterization of GP gestation (dams and fetuses) during chronic hypobaric hypoxia (CHH). Methods. Twenty-one GP were divided into normoxic (Nx, 11 dams, 27 fetuses) and hypoxic (Hx, 10 dams, 26 fetuses; hypobaric chamber at 3800m from day30 of pregnancy) groups. Doppler of uterine arteries (UtA) in pregnant GPs, middle cerebral artery (MCA), umbilical (UmbA), aorta, pulmonary, and hepatic arteries in fetuses were performed between 30-70 gestation days. Fetal biometric determinations such as biparietal diameter (DBP) and abdominal circumference (AC) were studied. Fetal heart morphology and systolic/diastolic function were determined since day40 of gestation. The variables were adjusted to best-fit function and compared by the extra sum-of-square F-test. All procedures were approved by the Local Bioethical Committee (CBA-1144-FMUCH). Results. Dams did not have UtA changes during pregnancy. However, Hx offspring showed smaller DBP and AC. The MCA pulsatility index showed a lower decrease in Hx compared to Nx fetuses. The UmbA has a higher resistance along with a higher cerebro-placental index in Hx relative to Nx. The heart of hypoxic fetuses was wider than controls. Right ventricular (RV) thickness in hypoxic fetuses has a decreased growth trajectory, with an increased size of the RV outflow tract. Systolic/diastolic functions of both ventricles were higher in the hypoxic group, as were the velocities of the aorta, pulmonary and hepatic arteries. Conclusions. CHH determines biometric, vascular, and functional changes during GPs pregnancies. The characterization of ultrasound changes during chronic hypoxic pregnancy may give clues to improve diagnosis, prognosis and treatment for a burden still without a treatment.

Financing: Funding. ANID 21191677, ANID 21230893, FONDECYT INICIO 11200798 and FONDECYT 1201283. Acknowledgments: Keywords. Chronic hypobaric hypoxia, fetal biometry, systolic/diastolic heart function.

Hypobaric hypoxia affects the aortic function during gestation and at birth in guinea pigs.

FA Beñaldo[1], AA Paz[1], T Jiménez[1], N Lorca[1,2], J Mocarquer[1,2], AA Candia[3], A González-Candia[3], EA Herrera[1]

(1) Universidad de Chile, Vascular Function & Reactivity Lab, Pathophysiology Program, ICBM, Faculty of Medicine, Santiago, Chile. (2) Universidad de Chile, Resident of Maternal-Fetal Medicine, Faculty of Medicine, Santiago, Chile. (3) Universidad de O'Higgins, Institute of Health Sciences, Rancagua, Chile

Background. Hypobaric hypoxia during pregnancy triggers responses in the cardiovascular (CV) system, which have a fundamental role in perinatal survival1. However, sustained physiologic responses may trigger long-lasting changes that increase CV risk. However, the relationship between fetal CV responses to hypoxia and the long-lasting effects is still scarce. Therefore, we propose to determine the aortic function by ultrasound during pregnancy and verify its vasoreactivity after birth in a guinea pig (GP) model exposed to hypobaric hypoxia during pregnancy. Methods. Twenty-one pregnant GP were divided into normoxic (Nx, n=11, 23 fetuses) and hypoxic (Hx, n=10, 27 fetuses, hypobaric chamber at 3800m) groups. Fetal aorta (FA) Doppler were performed between 30-70 days of gestation. Aorta wire myography was performed at birth. The groups were compared by extra sum-of-squares F test and Mann-Whitney t-test, considered significant when $p \leq 0.05$. All procedures were approved by the Local Bioethical Committee (20354-MED-UCH). Results. Fetal heart rate increased during pregnancy in Nx, while it remained constant in Hx. Aortic diameter increased during gestation in both groups. However, mean aortic blood velocity and velocity time integral (VTI) in Hx group increased during gestational age relative to Nx. In addition, neonatal aorta showed similar vascular responses to potassium, phenylephrine (vasoconstriction), and sodium nitroprusside (endothelium-independent vasodilator) in between groups. However, the vasodilation response to methacholine (endothelium-dependent) was lower in the Hx offspring. Conclusions. Our findings indicate that chronic hypobaria changes the aorta during fetal life, which program the neonatal aortic function towards a lower vasodilator capacity. We aim to follow the postnatal life of these animals to assess the long-lasting cardiovascular function.

Financing: Funding. ANID 21191677, ANID 21230893, FONDECYT INICIO 11200798 and FONDECYT 1201283.

Acknowledgments: Keywords. Hypobaric hypoxia, ultrasound, aorta vasoreactivity.

Consequences of gestational hypothyroxinemia over the hypothalamic-pituitary-adrenal axis activity in the offspring suffering ulcerative colitis

Esteban Caamaño[1,2], Enrique González-Madrid[1,2], María A. Rangel-Ramírez[1,2,3], Alejandro Regaldiz A.[1,2], Oscar Álvarez[1,2], M. José Mendoza-León[1,2], María Cecilia Opazo[2,4], Claudia Riedel[1,2]

(1) Universidad Andrés Bello, Ciencias Biológicas, Ciencias de la Vida, Santiago, Chile. (2) Instituto Milenio de Inmunología e Inmunoterapia., Santiago, Chile. (3) Instituto de Ciencias Biomédicas. Universidad Andrés Bello, Facultad de Medicina y Ciencias de la Vida, Santiago, Chile. (4) Instituto de Ciencias Naturales. Universidad de las Américas, Facultad de Medicina Veterinaria y Agronomía, Manuel Montt 948, Santiago, Chile

Background. Gestational hypothyroxinemia (HTX) is characterized by a reduction in the serum concentration of thyroxine (T4) with normal levels of triiodothyronine (T3) and thyroid-stimulating hormone (TSH). In human, and animal models gestational hypothyroxinemia (HTX) affects irreversibly the central nervous system (CNS). By using animal models, it has been shown that the offspring gestated in HTX (HTX-offspring) has a strong immune response. Given that corticosterone reduces immune response we believe that gestational HTX has been impaired the activity of the hypothalamus – pituitary – adrenal (HPA) axis in offspring. Methods. Pregnant C57BL6 mice were treated with 0.02% w/v methimazole (MMI) at embryonic day from 10 to 14, to induce HTX and obtain the first experimental group. The second group gestated under control conditions and received only tap-water. The offspring at postnatal 55 were treated with 2% sodium dextran sulphate (DSS) in drinking water for 7 days to induce ulcerative colitis (UC). Then mice were euthanatized and, the brain, colon, adrenal gland, and serum were obtained. The hypothalamus was dissected, and the content of corticotropin-releasing hormone (CRH) was analyzed in the hypothalamus by RT-qPCR. The content of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) hormones were analyzed in serum by ELISA. The mRNA of the enzyme 11β-HSD1 were analyzed in adrenal gland by RT-qPCR. The study was approved by the Andres Bello University ethics committee. Results. HTX-offspring showed reduced content of CRH mRNA in the hypothalamus and low levels of CORT in the serum. Even though, the HTX-offspring suffer intense UC compared to control offspring the levels of CRH, ACTH, CORT and 11β-HSD1 were similar to the offspring gestated in euthyroidism. Conclusions. Gestational HTX affects the function of the HPA axis by reducing the mRNA of CRH in the hypothalamus and the hormonal levels of CORT in the serum of its offspring.

Keywords. Hypothyroxinemia, glucocorticoids

Financing: Funding. ANID/Scholarship program/DOCTORADO NACIONAL/21202356. FONDECYT 1191300. AAEC 2023-2024. IMII ICN2021_045. Proyecto Núcleo UNAB DI-04-22/NUC.

Hypobaric hypoxia during second half of pregnancy alters placental function and fetal growth independently of HIFs in a guinea pig model.

Alejandro A. Candia[1,2], Tamara Jimenez[1], Felipe Beñaldo[1], Alejandro González-Candia[2], Bernardo J. Krause[2], Emilio A. Herrera[1]

(1) Vascular Function & Reactivity Lab, Pathophysiology Program, ICBM, Faculty of Medicine, Universidad de Chile., Chile. (2) Institute of Health Science, Universidad de O'Higgins, Rancagua, Chile

Background. Placental insufficiency is associated with pregnancy complications such as infections, preeclampsia, and maternal hypoxia. There is no treatment available and its study in human pregnancies is complex. The guinea pig is a reliable model of placental function1 and fetal programming2. Here, we aimed to determine in guinea pig, the placental and fetal outcomes in pregnancies exposed to hypobaric hypoxia. Methods. Pregnant guinea pigs were placed in a hypobaric chamber at near sea-level pressure (Control/C/n=13) or 480mmHg (Hx/n=7) from gestational day (G) 30 to its end. Umbilical artery (UA) and middle cerebral artery (MCA) pulsatility index (PI) were measured by Doppler ultrasound, and biparietal diameter (BPD) and abdominal circumference (AC) by echography. Cerebroplacental ratio (CPR) was determined as MCA to UA-PI. Animals were euthanized at G35 (G35C) or G60 (G60C and G60Hx) or naturally delivered (term~G70). Placentas were collected at G60 for immunohistochemistry of HIFs. Fetal and placental biometry were measured when the pregnancy resolved. Placental efficiency was determined as fetal-to-placental weight ratio. All procedures were approved by the Local Bioethical Committee (20354-MED-UCH). Results. UA-PI (quadratic fit) and MCA-PI (linear fit) decreased across pregnancy in both groups. UA-PI was higher in Hx. No differences were observed in CPR. At G60, placental efficiency was lower in Hx group, without significant differences in HIFs protein levels. BPD and AC increased across pregnancy (quadratic fit), with significatively lower values in Hx. Fetal weight and brain-to-fetal weight were decreased and increased, respectively, in Hx fetuses at G60. No differences were found in biometrical variables at postnatal day 1, however, gestational age at birth was higher in the Hx group. Conclusions. Hypobaric hypoxia induces placental insufficiency and fetal growth restriction across pregnancy in guinea pigs independently of HIFs changes. The guinea pig is a reliable model for Doppler ultrasound variables assessment in gestations with placental insufficiency.

Funding: CONICYT PFCHA/DOCTORADO NACIONAL/2019-21190352, FONDECYT 1201283 & FONDECYT 1220421.

References: 1. Candia AA et al. Vet Sci. 10(2):144, 20232. Morrison JL et al. J Physiol. 596(23):5535-5569, 2018

Intergenerational effect of prenatal androgen excess by the paternal line in a mouse model

Albert Carrasco Morales[1], Manuel Maliqueo Yevilao[2], Soledad Henríquez Barrera[3], Angélica López Troncoso[2], Gabriel Maldonado[4], Nicolás Crisosto King[2], Bárbara Echiburú López[2]

(1) Universidad de Concepción, Department of Animal Science, Faculty of Veterinary Science, Avenida Vicente Méndez 595, Chillán, Chile. (2) Universidad de Chile, Department of Internal Medicine, Faculty of Medicine, Carlos Schachtebeck 299, Interior Parque Quinta Normal, Santiago, Chile. (3) Universidad de Chile, Maternal and Child Research Institute, Faculty of Medicine, Avenida Santa Rosa 1234, Santiago, Chile. (4) Pontificia Universidad Católica de Chile, Department of Physiology, Endocrinology and Reproduction Unit, Avda. Libertador Bernando O'Higgins 340, Santiago, Chile

Androgen excess is clinically associated with polycystic ovarian syndrome, an endocrine-metabolic pathology, that is transmissible and perpetuable, from the mother to her female and male offspring. However, in a mouse model, offspring born to androgenized female mice and the effects on the next generation, conferred by the paternal line, have not been characterized. The aim was to evaluate the intergenerational effect of androgens on the offspring of males prenatally exposed to excess dihydrotestosterone (DHT) and determinate the effects of fed with a high fat diet (HFD) on F2. For this, 14-week-old (n=11) pregnant mice (C57BL/6) were injected daily with 50mL of a 250mg DHT solution for 3 consecutive days (16.5-18.5gd), while the control group received only the vehicle (ethanol, n=10). Males born from this protocol (F1), at 10 weeks of age, were crossed with control females (C=25; DHT=24). All procedures were approved by the bioethics committee. The resulting male offspring (F2) were fed with a control (CD=78) or HFD (n=36) from postnatal day21 and, at 10 weeks of age, they were euthanized for biometric and metabolic characterization. Androgenized mother mice (F0) tended to gain more weight during gestation (p=0.075) and had lower post-lactation insulin sensitivity compared to the control group (p=0.029). DHTF1-males were like the control group in biometric, metabolic and fertility parameters. At birth, F2-males were like controls and DHT F1-males, but we were able to demonstrate an effect of HFD compared to CD, both in the control group and DHT, causing an increase in body weight and a decrease in testicular and liver weight. Only in the control group we observed a greater weight of visceral and subcutaneous fat, induced by HFD. Preliminary data suggest that prenatal exposure to androgen excess does not cause biometric alterations in F2-males, however, we cannot rule out alterations in metabolic target tissues.

Keywords: Prenatal exposure to androgen excess, dihydrotestosterone, high-fat diet

Financing: FONDECYT Project No. 1201483.

"Distribution of folate forms in mothers and their offspring in relation to pregestational obesity"

Constanza Fuentes[1], Ana María Ronco[2], Paola Casanello[3], **Erika Castaño Moreno[2,4]**

(1) Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile. (2) Laboratory of Nutrition and Metabolic Regulation, INTA, University of Chile, Santiago, Chile. (3) Department of Neonatology and Department of Obstetrics, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile. (4) Tecnológico de Monterrey, Institute for Obesity Research, Monterrey 64849, NL, Mexico

Background. Low folate levels in pregnant women have been associated with pregestational obesity. Additionally, women with obesity have a higher risk of neural tube defects in their offspring, where the deficiency of folate plays an important role. This inverse relationship between folate levels and obesity before pregnancy would indicate a change in folate metabolism, with a decrease in plasma folate levels and an increase in erythrocyte folate1; however, there are no studies on how the distribution of folate forms in the mother and fetus is affected by maternal obesity. Methods. In pregnant women with term gestations, with pregestational overweight/obesity (BMI ≥25 Kg/m2) and healthy weight (BMI <25 Kg/m2) (n=81) and their offspring (n=152), were measured plasma total folate and RBC folate (5-MTHF, THF, 5,10-MTHF, 5-FTHF, UMFA, Mefox) by using LC/MS/MS. Data were analyzed by T-student and U-Mann-Whitney and considered significant when $p<0.05$. The study was approved by the institutional ethics committee. Results. The average pregestational BMI of the sample was 28.0 kg/m2, with a mean BMI of 32.7 kg/m2 for women classified as overweight/obese, indicating obesity. Pregnant women with a BMI ≥25 kg/m2 exhibited lower levels of erythrocyte folate (total folate, 5-MTHF, UMFA, THF, 5,10-MTHF, and Mefox; $p<0.05$). Additionally, newborns of mothers with a BMI ≥25 kg/m2 showed lower levels of plasma folate (total folate, 5-MTHF, UMFA, THF, 5-FTHF, and Mefox; $p<0.05$). The relative proportion of different forms of folate in the plasma varied according to the mother's BMI. Furthermore, the proportion of THF and 5,10-MTHF in the erythrocyte compartment was significantly higher in the umbilical cord than in maternal blood. Conclusions. Maternal pregestational BMI influences the concentration and distribution of folate forms in both maternal and offspring plasma and erythrocytes. The observed higher proportion of bioactive folate (THF) in the erythrocytes suggests an enhanced anabolism process in the fetus.

Keywords: Keywords. Pregestational obesity, plasma and RBC folate forms, cord blood

Association of maternal adipokines during pregnancy with one-month offspring´s Neurodevelopmental Outcomes

Andrea O. Castillo Santos[1], Miranda A. Molerés Orduña[1], Carla Arceo Cerna[1], Victoria Galicia Hernández[1], Guadalupe Estrada Gutiérrez[3], Jorge Valencia Ortega[2], Juan M. Solis Paredes[1], María del Carmen Hernández Chávez[4], Sandra Martínez Medina[4]

(1) Dept. of Perinatal and Rep. Health Research. (2) U. de Investigación en Rep. Humana, Facultad de Química, (4) Dept. of Developmental Neurobiology, (1,2,3,4) Instituto Nac. de Perinatología. Ciudad de México, Mexico

Background. Adipokines are pleiotropic molecules secreted by adipose tissue. It has been observed that the levels of adipokines throughout pregnancy are associated with neonatal anthropometric characteristics. Some adipokines affect the central nervous system, which explains their levels are associated with behavioral and neurodevelopmental disorders. This evidence suggests a possible association between maternal adipokine levels and neurodevelopmental alterations. Methods. From the prospective OBESO cohort of the National Institute of Perinatology, Mexico City, under the research and ethics committee grant numbers: 2019-1-20 and 3300-11402-01575-17, 97 healthy dyads of pregnant women and their term neonates were studied. Maternal pregestational body mass index (pBMI) was calculated, and maternal concentrations of progranulin, brain-derived neurotrophic factor (BDNF), adipocyte-specific fatty acid-binding protein (AFABP), fibroblast growth factor 21 (FGF-21), and adiponectin were quantified in each trimester of pregnancy by ELISA method. Child neurodevelopment was evaluated with Neonatal Behavioral Assessment Scale (NBAS) at one month. Binomial logistic regression was used to assess the association among maternal anthropometric characteristics, adipokines levels, and neonatal neurodevelopment. Results. After adjusting for pBMI, maternal age (β= 0.240, 95% IC: 1.007, 1.605 p= .044) and second trimester progranulin levels (β= -0.67, 95% IC: 0.884, 0.990, p = .021) were associated with orientation/interaction alterations. Third trimester BDNF levels (β= -.242, 95% IC: 0.641, 0.962, p= .019) were associated with state organization alterations. Meanwhile, third trimester AFAB levels (β= -.973, 95% (IC: 0.155, 0.920 p= .032) were associated with autonomic nervous system alterations. There was no association between maternal fibroblast growth factor-21 and adiponectin levels and the offspring´s neurodevelopmental outcomes. Conclusions. We show evidence that maternal adipokines play a significant role in child neurodevelopment. It is needed to elucidate its pathophysiology mechanism; however, these findings open a new research field about the role of maternal adipokines in fetal programming.

Keywords: Child neurodevelopment, Pregnancy, Adipokines, overweight/obesity

Financing: Funding. This study was funded by Instituto Nacional de Perinatología (grant numbers: 2019-1-20 and 3300-11402-01575-17)

Maternal high-fat diet impairs cognitive processes and hippocampal inhibitory synaptic transmission in mouse offspring.

Camila Cerna[1,2], Nicole Vidal[1,2], Gonzalo Cruz[1], Marco Fuenzalida[1,2]

(1) Center for Neurobiology and Integrative Physiopathology (CENFI), Institute of Physiology, Faculty of Sciences, University of Valparaíso, Valparaíso, Chile. (2) Neural Plasticity Lab, Institute of Physiology, Faculty of Sciences, Universidad de Valparaíso, Gran Bretaña 1111, Valparaíso, Chile

Background. Maternal obesity (MO) during pregnancy has become a significant global health concern. Extensive evidence suggests that maternal obesity during pregnancy can have enduring effects on the health and development of offspring. While metabolic and cardiovascular consequences of MO have been extensively studied, the impact on the nervous system, specifically cognition, is an emerging area. The hippocampus is particularly susceptible to insults during fetal development, including changes in nutrition. Previously it has been shown that a maternal high-fat diet (mHFD) leads to cognitive deficits in offspring, along with alterations in neurogenesis and the morphology of hippocampal neurons. However, the effect of this adverse prenatal environment on synaptic transmission has not been examined. Therefore, the main objective of our study was to investigate whether this detrimental prenatal environment impairs hippocampal synaptic transmission. Methods: We employed a mHFD, consisting of 60% of calories from fat. Female mice were exposed to the HFD for one month before pregnancy until lactation. To assess the cognitive function related to memory in the offspring, we employed the novel object recognition (NOR) and object localization memory (OLM) tests. Electrophysiological recordings were conducted in CA1 pyramidal neurons of the offspring mice to gain insights into the synaptic changes underlying the observed effects. Bioethics Approval CBC 26/2021. Results. Our study yielded significant findings indicating impaired cognitive performance in the offspring exposed to mHFD. This was evidenced by the discrimination index decrease observed in the novel object recognition (NOR) and object localization memory (OLM) tests. Furthermore, we discovered that mHFD led to an increase in inhibitory synaptic transmission. Conclusion. The findings from our study suggest that MO can lead to an increase in inhibitory synaptic efficacy within the hippocampus, resulting in an excitatory /inhibitory imbalance. These modifications may have implications for hippocampus-dependent cognitive function in offspring, providing valuable insights into potential mechanisms.

Keywords: Maternal High-Fat Diet, Neuroscience, Cognition

Financing: Funding. FONDECYT 1201816 (GC), ANID Fellowship 21202235 (CC).

Maternal plasma levels of benzo[a]pyrene and HMGB1 related to preterm birth.

Anyelo Durán[1], Ady Gioradano[2], Ivo Carrasco-Wong[1], Carolina Muñoz-Zamorano[3], Macarena Aguilera-Olguín[1], Sebastián E Illanes[4,5,6], Jaime Gutierrez[1], **Delia Indira Chiarello[1]**

(1) Universidad San Sebastián, Cellular Signaling and Differentiation Laboratory, School of Medical Technology, Faculty of Medicine and Science, Lota 2465, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, Inorganic Chemistry Department, Faculty of Chemistry and of Pharmacy, Santiago, Chile. (3) Universidad San Sebastián, PhD Program in Chronic Disease, Faculty of Medicine and Science, Lota 2465, Santiago, Chile. (4) Universidad de los Andes, Reproductive Biology Program, Center for Biomedical Research and Innovation (CiiB), Santiago, Chile. (5) Universidad de los Andes, Department of Obstetrics and Gynecology, Faculty of Medicine, Santiago, Chile. (6) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background. The ubiquity of exposure to polycyclic aromatic hydrocarbons (PAHs) has led to great interest in understanding their effects on pregnancy outcomes. Recent studies have indicated that PAHs may trigger preterm delivery by inducing an inflammatory response. Our aim was to explore the correlation between a PAH metabolite, benzo(a)pyrene, and a proinflammatory factor such as high mobility group box 1 protein (HMGB1), on pregnancy outcome. Methods. Pregnant women participating in the study (57) were volunteers recruited from the patient population of the Universidad de los Andes Clinic in the city of Santiago, Chile. The concentration of benzo(a)pyrene was determined using a gas chromatograph–mass spectrometer (PerkinElmer Clarus 680) coupled with a mass spectrometry instrument. HMGB1 was measured by ELISA kit of Novus Biological. The statistical analyses were conducted using Graphpad Prism 9. All participants gave written informed consent, and the study protocol was approved by the Scientific Ethics Committee of the Universidad de los Andes (Chile) (ethical code: CEC2022058). Results. Benzo(a)pyrene levels are increased ($p<0.05$) in pregnant women who delivered prematurely compared to term pregnancies. At the same time, a positive correlation was found ($p<0.05$; $r=0.8412$) between Benzo(a)pyrene and HMGB1 levels in plasma of pregnant women with preterm delivery. Conclusions. Further studies are needed to understand the relationship between increased benzo(a)pyrene and HMGB1 in preterm labor.

Keywords: Preterm birth, Benzo(a)pyrene, HMGB1, Exposome Plasma benzopyrene levels in pregnant women at term and preterm delivery. Correlation between maternal benzopyrene levels and HMGB1 in preterm labor.

Financing: CONICYT, PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2019, Folio N°PAI77190060 to DC. Fondecyt Regular #1221362, Chile to JG. Acknowledgments: Patricia Valdebenito, midwife at the Universidad de los Andes Clinic.

Prevalence of stunting and its association with complementary feeding indicators in children aged 6-23 months.

Giovana Nigri Cursino[1], Dayana Rodrigues Farias[1], Raquel Machado Schincaglia[1], Maria Beatriz Trindade de Castro[1]

(1) Federal University of Rio de Janeiro, Social and Applied Nutrition, Institute of Nutrition, Av. Carlos Chagas Filho, 373 - Bloco J, 2° andar, sala 21, Rio de Janeiro, Brazil

Background. Stunting has short- and long-term consequences, including impairments in child growth and development1. Thus, the aim of this study was to estimate the prevalence of stunting among Brazilian children receiving primary health care and to analyze its association with the indicators of complementary feeding. Methods. This cross-sectional study used data from 119.848 children aged 6-23 months registered at the Brazilian Food and Nutrition Surveillance System (SISVAN) in 2019. In the case of repeated measures, only the last measurement of length (cm) and dietary food markers (referring to the day before the interview) of each child were used. Stunting was classified based on the length-for-Age z score, according to WHOrecommendations2. The following indicators of complementary feeding practice were assessed: consumption of iron-rich foods, unhealthy foods, sugar beverages and zero vegetables or fruits3,4. The analysis included the estimation of prevalence (%) and their 95% confidence interval (95%CI) and logistic regression models adjusted for geographic regions, sex, participation in the National cash transfer program – Bolsa Família (BPF), and child age. Results. The prevalence of stunting was 13.3% (95%CI:13.1;13.5). The highest prevalence was observed in males (15.1%), residents in the North macro-region (20.1%), and BPF participants (16.8%). Iron-rich food consumption (OR=0.77; 95%CI: 0.72;0.81) was associated with lower odds of stunting. Children with zero vegetables or fruits consumption (OR=1.32; 95%CI:1.24;1.41) had higher odds of stunting, compared to those who consumed fruits and vegetables on the day before. Conclusions. The study found a high prevalence of stunting among children aged 6-23 months, and this condition was associated with complementary feeding indicators. The results support the knowledge that an adequate complementary feeding introduction can play a protective role against stunting in children aged 6-23 months. Ethical approval: The study was approved by the Ethics Committee of the IPPMG, UFRJ (CAAE: 18447919.0000.5264).

Keywords: Malnutrition, Stunting, Food consumption

Financing: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES – Brazil (CAPES); Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ – Brazil.

Association of metabolome at pregnancy and infant growth throughout the first year: a Brazilian cohort

Samary Da Silva Rosa Freire[1], Marina Padilha[1], Ana L. Lima Ferreira[1], Raquel Machado Schincaglia[1], Amanda C. Cunha Figueiredo[1], Nathalia C. Freitas-Costa[1], Gilberto Kac[1]

(1) Nutritional Epidemiology Observatory, Josué de Castro Nutrition Institute, Rio de Janeiro Federal University, Rio de Janeiro, Brazil

Background: The first 1000 days of life are a pivotal period for development and growth. Examining the metabolome during this phase allows to gain insights into the metabolic profile that drive infant growth(1, 2). The study aimed to explore the association between the maternal metabolome in the third trimester of pregnancy and infant growth during the first year. Methods: A prospective cohort of 95 healthy mother-infant dyads. Maternal blood sample was collected at pregnancy (28-35 gestational weeks - baseline) for target metabolome analysis, which identified 132 metabolites including amino acids, biogenic amines, acylcarnitines, lysophosphatidylcholines(LPC), diacyl-phosphatidylcholines(PC), alkyl:acyl-phosphatidylcholines(PC-O), sphingomyelins, and hexoses. The infant growth was assessed with weight-for-age, length/height-for-age, weight-for-length/height, Body Mass Index (BMI)-for-age, and head circumference-for-age indexes, at 1, 6 and 12 months of life. Linear mixed-effects models were performed to explore associations between maternal metabolome and infant growth. All models were adjusted for confounders [maternal pre-pregnancy BMI, gestational age at birth, and infant birth weight]. Adjustments for false discovery rate were considered [p-value < 0.05 (q-value)]. The study has been approved by two Research Ethical Committee. Results: 55.3% of participants self-reported as brown/mixed skin color, 42.11% were primiparous, and the median birth weight was 3262.5 g (IQR=3020-3605). A directly association was found between the z-score of weight-for-age and lysoPCa 24:0 (β = 0.0019; q-value = 0.01), weight-for-length/height and taurine (β=0.002; q-value =0.0008), alanine (β=0.001; q-value =0.01), ornitine (β=0.002; q-value =0.02) and asymmetric dimethylarginine (β=0.001; q-value =0.04), of BMI-for-age and taurine (β=0.001; q-value =0.02) and ornitine (β=0.001; q-value =0.04) and of head circumference-for-age and trans-hydroxyproline (β=0.003; q-value =0.02) and a inversely association with valine (β=-0.002; q-value =0.03), PC 36:3 and 38:3 (β=-0.003; q-value =0.02), and PCO 38:3 (β=-0.003; q-value =0.02). Conclusion: The results revealed that differences in metabolome at the end of pregnancy could influence the growth outcomes of the offspring.

Keywords: Metabolome, pregnancy, infant growth

Financing: CAPES, CNPq and FAPERJ. Acknowledgments: We thank all participants and their children from the cohort, and the Rio de Janeiro Public Health Center, where the data was collected.

Overexpression of connexin 43 induced neuronal apoptosis in the cerebral cortex of the neonates gestated in intrauterine hypoxia.

Esteban Figueroa[1], Adolfo Paz[2], Emilio A Herrera[2], Alejandro Gonzalez Candia[1]

(1) University of O'Higgins, Rancagua, Laboratory of Fetal Neuroprogramming, Institute of Health Sciences, Libertador Bernardo O'Higgins 611, Rancagua, O'Higgins, Rancagua, Chile. (2) Universidad de Chile, Vascular Function & Reactivity Laboratory, Faculty of Medicine, Salvador 486, Providencia, Santiago, Chile

Background. Intrauterine hypoxia, a condition of reduced oxygen supply during fetal development, has been associated with adverse neurological outcomes1. Connexin 43 (Cx43), a protein involved in intercellular communication, plays a critical role in neuronal function and survival2. This study aims to investigate the impact of intrauterine hypoxia on Cx43 expression and its possible correlation with neuronal cell death in the cortex of Guinea pig neonates, providing insight into the underlying factors contributing to neurological deficits associated with hypoxic gestation.

Methods. All animal care, procedures, and experiments were approved by the Institutional Committee (Certificate 20418-MED-UCH). We assigned five newborn Guinea Pigs to the normoxia group (Nx) and five newborn Guinea Pigs to the hypobaric hypoxia group (Hx). On gestational day 30 (GD30), both groups were exposed to a hypobaric chamber under normoxia conditions (Nx, controls, 720 torrs, ~580 m) or hypoxia conditions (Hx, 470 torrs, ~3800 m) until delivery. On GD70, the animals were euthanized, and fetal brain samples were collected. The protein expression related to Cx43 and Caspase 3 cleavage was evaluated using western blot and immunohistochemistry techniques in the brain. For neuronal density studies, staining was performed with toluidine, and TUNEL assay kits evaluated apoptosis. The results were analyzed using a nonparametric student t-test. Statistical significance was considered when $p<0.05$.

Results. Although no changes in total brain tissue expression of connexin 43 and caspase 3 cleavage were observed, immunolocalization of these proteins in neurons of the cerebral cortex showed a significant increase in connexin 43 and caspase 3 in the Hx group compared to Nx, which is also related to a lower neuronal density and increased of neuronal apoptosis in the cortex of neonates gestated in hypoxia.

Conclusions. Intrauterine hypoxia up-regulated Connexin 43 expression induces neuronal death in the neonatal brain.

Keywords: Connexin 43, Neuroendothelium, Gestational Hypoxia

Financing: Fondecyt de Inicio 11200798 and Fondecyt Regular 1201283

Maternal levels of progranulin and brain-derived neurotrophic factor as predictors of birth weight

Carla Arceo Cerna[1], **Victoria Galicia Hernández[1]**, Miranda Adriana Molerés Orduña[1], Andrea Ofelia Castillo Sanchez[1], Jorge Valencia Ortega[2], Otilia Perichart Perera[3], Carolina Rodríguez Hernández[3], Guadalupe Estrada Gutierrez[4], Juan Mario Solis Paredes[1]

(1) Instituto Nacional de Perinatología, Department of Reproductive and Perinatal Health Research, Mexico City, Mexico. (2) Instituto Nacional de Perinatología, Unidad de Investigación en Reproducción Humana, Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico. (3) Instituto Nacional de Perinatología, Department of Nutrition and Bioprogramming, Mexico City, Mexico. (4) Instituto Nacional de Perinatología, Research Division, Mexico City, Mexico

Background: Adipose tissue plays an important role in the production of adipokines. These participate in physiological processes such as glucose and lipid metabolism and reproductive processes such as placentation and fetal growth. However, it is still unknown whether maternal concentrations of these adipokines are associated with the anthropometric characteristics of the neonate. Methods: Under follow-up within the OBESO (Biochemical and Epigenetic Origin of Overweight and Obesity), under the research and ethics committee grant numbers: 2019-1-20 and 3300-11402-01575-17) perinatal cohort (Instituto Nacional de Perinatología), pregnant healthy women were recruited (n=98). Maternal serum levels of progranulin, brain-derived neurotrophic factor (BDNF), fibroblast growth factor 21 (FGF-21), adiponectin, and adipocyte-specific fatty acid-binding protein (AFABP) were quantified by ELISA during the three trimesters of pregnancy. A multiple linear regression model was performed to evaluate the association between age, pregestational body mass index, gestational weight gain, and maternal adipokines with birth weight, using SPSS v.27 software. Results: The multiple linear regression model significantly explained birth weight, having an R^2= 0.710 and adjusted R^2= 0.65. The variables that explained the model were maternal age [β= -0.37 (95% CI: -0.055, -0.019, p= .001)], progranulin [β= 0.005 (95% CI: 0.003, 0.008, p= <.0001)] and BDNF [β= 0.017 (95% CI: 0.003, 0.032, p= .020)] in the third trimester of pregnancy. Conclusions: Our findings indicate that neonatal weight is influenced by maternal progranulin and BDNF in the third trimester of pregnancy and by maternal age. These results highlight the importance of considering adipokines in the assessment and understanding of fetal development as well as their involvement in fetal programming processes.

Keywords: pregnancy, birth weight, progranulin, BNDF

Financing: This study was funded by Instituto Nacional de Perinatología (grant numbers: 2019-1-20 and 3300-11402-01575-17)

Butyrate improves offspring liver metabolic programming in a maternal overweight rat model

Florencia Heinecke[1], Marina Labiano[1], Jeremías Pablo Flores Quiroga[1], María Agustina Meneghini[1], Alicia Graciela Faletti[1], Verónica White[1]

(1) CEFYBO-CONICET-UBA, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Ciudad Autónoma de Buenos Aires, Argentina

In a rat model of maternal overweight, we previously observed that maternal oral administration of Butyrate, a postbiotic from intestinal microbiota, prevented overgrowth and liver lipid overaccumulation in fetuses; while in mothers showed indicators of an increase in insulin sensitivity. Our aim was to evaluate whether butyrate was also able to ameliorate the negative program induced by maternal overweight in the offspring and to improve maternal metabolic parameters.

Methods: Female Wistar rats were fed standard (CT rats) or saturated fat-rich-diet (FD rats) for 8 weeks and mated with control males. Butyrate (3%) or vehicle was orally delivered daily during gestation and 3 days per week during lactation (FDB rats). The mothers were euthanized after weaning and the offspring at 140 days of life. Offspring were fed with control diet. Maternal and adult offspring liver levels of triglycerides (TG) and cholesterol esters (CE) were assessed by TLC, lipoperoxidation by TBARS assay, mRNA levels by RT-qPCR, and hepatic enzymes (ALT and AST) circulating activity by IFCC method. CICUAL of the Faculty of Medicne UBA approved by resolution n°4081/04, year 2022.

Results: FD mothers and offspring showed an increase in adipose mass that was not observed in the FDB group. Maternal liver showed overaccumulation of TG, CE and increased levels of Srbp-1c and lipoperoxidation levels, the latter prevented by Butyrate. Butyrate also prevented the increase in ALT activity observed in FD maternal serum. Female and male offspring displayed increased adipose mass, liver TG overaccumulation and increased ALT and AST circulating levels, all prevented by Butyrate.

Conclusions: Maternal overweight induced an increase in lipid overaccumulation and liver tissue damage indicators in both mothers and offspring. In the offspring, butyrate prevented liver tissue damage and lipid overaccumulation, while in the mothers it also prevented liver tissue damage but lipid overaccumulation persisted maybe due to the increased Srebp-1c mRNA levels.

Keywords: Maternal overweight, Intrauterine programming, Butyrate

Financing: Préstamos BID PICT 2020 Serie A nº 01125 y PICT 2021 nº I-A-00281, CONICET

Supplementing obese rats with DHA before pregnancy and throughout lactation reduces metabolic dysfunction in mothers and offspring

Guadalupe Leticia Rodríguez González[1], Luis A Reyes-Castro[1], **Carlos A Ibañez[1]**, Gimena Juárez-Pilares[1], Armando A Arredondo-López[2], Fengyang Huang[3], Francisco Bolaños-Jiménez[4], Elena Zambrano[1]

(1) Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Reproductive Biology, Mexico City, Mexico. (2) Instituto Nacional de Salud Pública, Cuernavaca, Mexico. (3) Hospital Infantil de México Federico Gómez, Mexico City, Mexico. (4) Université de Nantes, INRA, UMR1280 Physiologie des Adaptations Nutritionnelles, Nantes, France

Background. Obesity during pregnancy increases the risk of short- and long-term metabolic dysfunction in the mother and her offspring and later obesity (1). Dietary patterns in obese individuals contribute to a reduction in docosahexaenoic acid (DHA), which may exacerbate adipose tissue development and metabolic alterations (2). We aimed to determine if DHA supplementation prior and throughout pregnancy and lactation to obese rats improves both maternal and offspring adiposity and metabolism. Methods. The Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán Animal Experimentation Ethics Committee (BRE-1870) approved all procedures. Female rats ate control (C: 5%-fat) or obesogenic diet (MO: 25%-fat) from weaning to lactation. One month before mating and during pregnancy and lactation, rats received orally 400 mg/kg/day of DHA (C+DHA and MO+DHA) and remained on their respective diet. After weaning offspring ate C diet until postnatal day 110. Body weight, total fat, adiposity index, cholesterol, triglycerides, leptin, glucose, insulin concentrations and insulin resistance index were determined in both the mothers (end of lactation) and the offspring. Results. MO mothers, and male and female offspring had increased weight, total fat, and adiposity index, whereas body weight, total fat and adiposity index were partially reduced in the mother and their offspring. Triglycerides serum levels were higher in MO mothers and male and female offspring, which were in part decreased by maternal DHA supplementation (MO+DHA). Leptin serum levels were higher in both MO and MO+DHA mothers and male and female offspring. Insulin serum levels and insulin resistance index were higher in MO mothers and male and female offspring; and were partially decreased by maternal DHA supplementation (MO+DHA). Cholesterol and glucose levels were similar among the groups. Conclusions. Maternal DHA supplementation attenuated in both maternal and young adult offspring the excessive fat accumulation and the metabolic alterations caused by maternal obesity. 1.PMID:31591717, 2.PMID:29621669

Keywords: DHA, METABOLISM, PROGRAMMING, INTERVENTIONS, OBESITY

Financing: ANR-CONACyT (Agence Nationale de la Recherche, Consejo Nacional de Ciencia y Tecnología) 2015-16-273510

Resveratrol supplementation before and through pregnancy in obese rats leads to sex-specific placental responses

Carlos Alberto Ibáñez Chávez[1], Lilia Vargas-Hernández[1,2], Guadalupe L Rodríguez-González[1], Luis A Reyes-Castro[1], Claudia J Bautista-Carbajal[1], Consuelo Lomas-Soria[1], Elena Zambrano-González[1]

(1) Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Departamento de Biología de la Reproducción, Vasco de Quiroga 15, Belisario Domínguez Secc XVI, Tlalpan, 14080, Ciudad de México, México. (2) Instituto Mexicano del Seguro Social, Hospital de Ginecología y Obstetricia No.4 Luis Castelazo Ayala, Ciudad de México, México

Background. Maternal obesity leads to intrauterine growth restriction. Placental physiology is fundamental for fetal growth. Maternal resveratrol supplementation counterbalanced rat placental oxidative stress in obese mothers (1). Placental development has sex-specific trajectories (2) with different potential responses to the prenatal environment. We aimed to determine the effects of maternal obesity and resveratrol supplementation on male and female placental efficiency (PE) and ultrastructure. Methods. Female Wistar rats (F0) were weaned to chow (C) or a high-fat diet (MO). One month before mating until 19 days' gestation (dG), half the F0 received 20 mg resveratrol/kg/d orally (CRes and MORes). All F0 were euthanized at 19 dG and immediately fetuses were euthanized, sexed, and placentas weighed. Fetal weight, PE (as fetal/placental mass ratio), and placental ultrastructure (by blood vessel and villus size in histology slides) were analyzed by 2W-ANOVA within males and females. Mean ± SEM, P <0.05 (*) for C vs MO and CRes vs MORes and different letters for C vs Cres and MO vs MORes. Procedures approved by INCMNSZ Animal Experimentation Ethics Committee. Results. Fetal weight (g) was decreased in MO and MORes but increased in CRes vs C in both, males (C=2.6±0.1a, CRes=2.9±0.1b, MO=2.2±0.1* and MORes=2.3±0.1*) and females (C=2.4±0.1a, CRes=2.7±0.04b, MO=2.2±0.1* and MORes=2.2±0.1*). PE (Fig. 1 D) and placental villus size (Fig. 1 E) were increased in female Cres vs C. Placental blood vessel size was increased in male MO vs C (Fig. 1 C). Conclusions. Maternal obesity in the rat leads to fetal growth restriction in males and females with alterations in placental circulation in males. Maternal resveratrol intervention increases the placental efficiency with placental ultra-structural changes in females. Different placental alterations in response to maternal obesity mediate fetal growth restriction in a sex-specific manner.

Keywords: Maternal obesity, resveratrol, placental efficiency

Financing: Newton Fund RCUK-CONACyT (Research Councils UK—Consejo Nacional de Ciencia y Tecnología). I000/726/2016 FONCICYT/49/2016

Determination of maternal supraphysiological hypercholesterolemia early in pregnancy, its consequences in cardiovascular risk markers and intima media thickness

Ramón Serra[1,2], Gloria Bravo[1], Claudette Cantin[3], Sebastián Illanes[2,4,5], **Andrea Leiva[3]**

(1) Hospital Naval, Punta Arenas, Chile. (2) Universidad de Los Andes, Facultad de Medicina, Santiago, Chile. (3) Universidad San Sebastián, Facultad de Medicina y Ciencia, Santiago, Chile. (4) Universidad de los Andes, Laboratory of Reproductive Biology, Center for Biomedical Research and Innovation (CIIB), Santiago, Chile. (5) Universidad de los Andes, IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background: Maternal supraphysiological hypercholesteremia (MSPH) occurs in ~20% of human pregnancies, leading to increased maternal pro-atherogenic LDL, fetoplacental endothelial dysfunction, oxidative stress, fetal atherosclerosis, and cardiovascular disease (CVD) later in life (1-4). Nevertheless, HC in pregnancy is not clinically determined and there are no strategies for early detection and control of its deleterious effects. Aim: To assay early determination of MSPH as well as its association with cardiovascular risk markers and intima media thickness (IMT) in the maternal and fetal vasculature. Methods: Prospective cohort: 46 women were recruited at trimester (T) 3 and followed until delivery (ethics ID2021051, U. Andes). Lipid profile, apolipoprotein A and B, oxidized LDL and lipid peroxidation (MDA) were measured in maternal and umbilical cord serum at delivery. Maternal carotid and fetal aortic IMT were determined by ultrasonography at T3. Retrospective cohort: Lipid profile was determined at T1 and T3 in 66 pregnant women. With biochemical parameters was elaborated a ROC curve for development of an algorithm to determine MSPH at T1. Data were analyzed by t-test ($p<0.05$). Results: The ROC curve indicated that women who will develop MSPH at T3 can be identified at T1 with a specificity of 97% and sensibility of 95% by probing combinations of classical biochemical parameters of T1. In MSPH women were determined increased CVD risk markers (total and LDL cholesterol, oxLDL, MDA and ApoB/ApoAI ratio at delivery) compared to control pregnancies. In MSPH neonates, oxLDL and MDA were increased compared to control neonates. Nevertheless, maternal carotid and fetal aortic IMT was similar in the MSPH and control groups. Conclusion: We developed an algorithm to detect at T1 the 97% of the women that will develop MSPH. MSPH women and neonates had increased CVD risk markers; however, specific vascular damage was absent at delivery time, suggesting a window for interventions.

Keywords: Hypercholesterolemia in pregnancy, cardiovascular disease risk markers, intima media thickness

Financing: Funding: Fondecyt 1230527. Basal Funding for Scientific and Technological Center of Excelence, IMPACT, #FB210024.

Polyphenol-enriched-extra-virgin-olive-oil (EVOO) improves vascular dysfunction and oxidative stress in a model of hypercholesterolemia in pregnancy

Blanco Alba[1,2], Albistegui Rodrigo[1], Alvaro Santana[2], Pablo Espinoza[2], Claudia Reyes[2], Andrea Morales Garrido[3], Jaime Gutierrez[3], Alfonso Mate[2], Carmen Vazquez[2], **Andrea Leiva[3]**

Universidad de Sevilla, (1) Máster en Fisiología y Neurociencias y (2) Departamento de Fisiología, Facultad de Farmacia, Sevilla, España. (3) Universidad San Sebastián, Facultad de Medicina y Ciencia, Santiago, Chile

Background: Maternal supraphysiological hypercholesteremia (HC) occurs in ~20% of human pregnancies associating with increased pro-atherogenic LDL and cardiovascular risk markers in the mother/neonate, fetoplacental endothelial dysfunction, oxidative stress, fetal atherosclerosis, and cardiovascular disease later in life (1-4). Nevertheless, HC in pregnancy is not clinically determined and there are no interventions to improve its deleterious effects. EVOO reduces oxidative stress in cardiovascular patients and is secure in pregnancy; however, its effects on maternal HC are unknown (5). Aim: To determine the effect of EVOO on endothelial function and oxidative status in a model of maternal HC. Methods: Pregnant C57BL/6 mice were aleatorized to control (chow diet), HC (diet 2% cholesterol + 0,5% cholate, from gestational (GD) day 13 to 18) or HC+EVOO groups (HC diet + 12% EVOO from GD 13 to 18). After euthanasia, maternal total cholesterol (TCh), endothelial function, hepatic oxidative status, and NADPH activity were evaluated. In the fetus, TC and soluble markers of endothelial dysfunction (VACAM, ICAM) were determined (ethic ID 13/03/2019/031, Junta de Andalusia, España). Only significant differences are showed ($p<0,05$). Results: HC and HC+EVOO dams presented increased TCh (151,5 and 128,3 vs 51,2 mg/dL, respectively) and liver/weight ratio (5,7 and 6,3 vs 4,8%, respectively) compared to control group. In HC dams, hepatic DHE staining, and NADPH activity was increased (301,3 vs 100 and 194,7%, respectively) compared to C and HC+EVOO groups meanwhile methacholine-mediated aortic relaxation was reduced (52,3 vs 76,4 and 82,3%, respectively). In the fetus, placental efficiency was similar between the groups. Remarkably, TCh (0,64 vs 0,2 ug CT/ug fetal tissue), VCAM (13,1 vs 9,9 ng/mL) and ICAM (30,1 vs 20,9 ng/dL) levels were higher in HC compared to HC+EVOO offspring. Conclusion: EVOO supplementation at end of pregnancy improves vascular dysfunction and oxidative stress in a mice model of pregnancy-HC.

Keywords: Hypercholesterolemia in pregnancy, endothelial dysfunction, oxidative stress

Funding: Fondecyt 1230527, 1221362 and Beca Fundación Carolina estancias cortas.

A comparison of methods for the isolation of extracellular vesicles from pregnancy woman plasma.

Carla Leiva[1,2], Belén Gaete-Ramírez[3], Manuel Varas-Godoy[3], Manuel Maliqueo[1]

(1) Universidad de Chile, Endocrinología, Medicina, Parque Quinta Normal, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, Ciencias de la Salud / Carrera de Nutrición y Dietética, Medicina, Vicuña Mackenna 4860, Santaigo, Chile. (3) Universidad San Sebastián, Cancer Cell Biology Lab., Centro de Biología Celular y Biomedicina (CEBICEM), Medicina y Ciencia, Los Leones-Lota 2465, Santiago, Chile

Background. Extracellular vesicles (EVs) are nanometer-sized vesicles containing nucleic acids and proteins that are released from a multitude of cell types and have gained significant interest as potential diagnostic biomarkers. In this sense, the EVs found in maternal plasma could constitute early markers of pregnancy pathologies and be a means of communication and maternal-fetal interaction. Therefore, it is relevant to have methods that allow us to isolate electric vehicles with high yield and purity. Our aim was to compare two EV isolation methods: ultracentrifugation (UC) and size exclusion chromatography (SEC).

Methods. EVs were isolated from one milliliter of plasma from three pregnant women, by UC and SEC. The size and yield of the particles isolated by each method was determined by nanoparticle tracking analysis (NTA). The presence of exosome markers (Alix, CD63 and TSG101) and lipoproteins (APOB) was determined by western blot. The study was approved by the committee of University of Chile 201-2017.

Results. It was observed that the particles isolated by UC showed a greater modal size than those from SEC (122.0 ± 12.9 nm vs 75.9 ± 4.3 nm, $P < 0.05$). Regarding the isolation of particles between 50 - 150 nm that would correspond to EVs, the SEC method had a higher performance than the UC method (2.77 x 1011 ± 9.29 x 1010 vs 6.39 x107 ± 9.99 x 106, $P < 0.05$). The SEC fraction containing the EVs expressed the exosome markers Alix, CD63 and TSG101. However, some presence of APOB was detected in the samples isolated by SEC.

Conclusions. The EVs isolated with SEC method were characterized by a high yield, however the method must be optimized to obtain a fraction with less lipoprotein contamination. Therefore, SEC seems to be an efficient method for the isolation of EVs isolated from maternal plasma.

Keywords: Extracellular vesicles, pregnancy, isolation methods

Financing: ANID Becas/Doctorado Nacional 21220507.

PPARγ methylation and expression in neonatal monocytes from women with pregestational obesity supplemented with DHA

Macarena Lépez[1,2], Torsten Plösch[3], Cristina Silva[4], José Antonio Castro-Rodríguez[5], María Luisa Garmendia[6], Paola Casanello[7,8]

(1) P. Universidad Católica de Chile (PUC), PhD in Medical Sciences, Santiago, Chile. (2) Universidad de Chile, Faculty of Medicine, Santiago, Chile. (3) University Medical Centre Groningen (UMCG), Groningen, The Netherlands. (4) U. San Sebastian, PhD in Cell Biology and Biomedicine, Santiago, Chile. (5) PUC, Division of Pediatrics, FM, Santiago, Chile. (6) Universidad de Chile, INTA, Santiago, Chile. (7) PUC, Department of Neonatology, FM, Santiago, Chile. (8) PUC, Department of Obstetrics, FM, Santiago, Chile

Background. The offspring of women with pregestational obesity (PGO) have an impaired immune function in their postnatal life (1), where PPARγ has an essential role in immune modulation (2). Docosahexaenoic acid (DHA) supplementation during pregnancy decreases systemic inflammation in PGO (3), but its molecular mechanism is still not fully understood. This study aimed to determine the effect of DHA supplementation during pregnancy on PPARγ promoter DNA methylation (DNAm) levels and transcripts in umbilical cord blood monocytes (UCB-Mo) from the offspring of women with PGO. Methods. UCB-Mo DNA and RNA were purified from the offspring of PGO and normal- weight (NW), with (200 and 800 mg/d) and without DHA supplementation during pregnancy (ASMA and EpiFat Studies). Samples were separated into groups: NW, PGO, PGO-200, PGO-800. Global DNAm of PPARγ were analyzed by Illumina 850K array, and five methylation sites (CpG sites) were quantified by pyrosequencing (PyroMark Q24, Qiagen). RNA transcript of PPARγ was quantified by RT-qPCR. Results. There was an increase in the number of differentially methylated probes (DMPs) of the PPARγ promoter in UCB-Mo of PGO-800 vs PGO, in relation to PGO vs NW ($p<0.05$). DNAm levels in CpG3 (-339 from transcription start site) of PPARγ increased by ~2% in PGO or PGO-200 vs NW (~9% vs ~7%, $p=0.021$ and 0.044, respectively), and decreased in PGO-800 vs PGO or PGO-200 ($p=0.009$). These changes were associated with an increase in the levels of PPARγ transcripts (PGO-200 vs PGO-800, $p=0.007$). Conclusions. PGO is associated with a decreased expression and increased DNAm levels of PPARγ in the immune cells of the offspring, which could be related to increased neonatal inflammation. Maternal supplementation with 800 mg/d of DHA can reverse these changes, suggesting beneficial effects of polyunsaturated fatty acids in the offspring born to women with PGO. References. 1) DOI: 10.4049/jimmunol.1700434; 2) DOI: 10.1016/j.bbalip.2007.02.005; 3) DOI: 10.3390/nu13010247. Keywords: pregestational obesity, cord blood monocytes, PPARγ DNA methylation.

Funding: DOHaD Brain Mobility award 2021 (ML), Fondecyt 1171406 & 1221812 (PC), Fondecyt 1141195 (JAC), Fondecyt 1150878 (MLG), SOCHED 2021-11 (PC, ML).

Metformin improves metabolic dysfunction induced by early overnutrition in Balb/c mice

Ananda Malta[1,2], Lucas Saavedra[1,2], Douglas Almeida[1], Scarlett Raposo[1], Willian Rodrigues[1], Letícia Ferreira[1], Paulo Mathias[1]

(1) State University of Maringa, Laboratory of Secretion Cell Biology, Department of Biotechnology, Genetics and Cell Biology, Colombo avenue, 5790., Maringá, Brazil. (2) Institute of Applied Research in Medicine-INSPAM, Nestor Guimaraes, 281, Ponta Grossa, Brazil

Background: Overfeeding during lactation causes early obesity, hyperphagia, and glucose dyshomeostasis throughout life. Metformin is an antidiabetic medication that in addition to the effects of lowering blood glucose, it seems to have an impact on reducing food intake, body weight, and, improved metabolism. In this way, we evaluated the metformin effects on metabolic dysfunction in treated adult obese mice, induced by postnatal overnutrition.

Methods: All experimental protocols were performed in accordance with the standards of the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee of the State University of Maringa (CEUA), protocol no. 8137280920. After birth, male BALB/c mice were raised in small litters (SLs, 2 pups/dam, n=9) and normal litters (NLs, 6 pups/dam, n=8) to obtain early overfeeding and normal feeding during lactation. Offspring groups were weaned on day 21. At 120 days of age, a subset of SL mice received metformin treatment (Multivida; 100mg/kg bw) in their drinking water until 180 days of age. Control NL and SL mice received normal drinking water. Body weight and food intake were assessed during the experimental protocol. Body composition, food intake, glucose tolerance, and lipid profile were evaluated at 180 days of age. Data were analyzed by one-way ANOVA and considered significant when $p<0.05$.

Results: Early overnutrition induced by small litter during lactation caused metabolic dysfunction in SL mice in adulthood. The metformin treatment reduced body weight, adiposity, food intake, triglycerides, and cholesterol blood levels, and improved glucose tolerance in SLM mice.

Conclusions: Our data indicate the therapeutic benefits of metformin on appetite, body mass, and glucose tolerance in the obesity phenotype promoted by early overnutrition. Further studies will be required to determine the mechanisms involved.

Financing: National Council for Scientific and Technological Development – CNPqInstitute of Applied Research in Medicine - INSPAMJBS S.A Company.

Late postnatal effects of hypobaric hypoxia during gestation on the carotid artery in guinea pigs

Cristian Marambio[1], Adolfo Paz[1], Felipe Beñaldo[1], Tamara Jimenez[1], Emilio Herrera[1,2]

(1) Universidad de Chile, Laboratorio de Función y Reactividad Vascular, ICBM, Facultad de Medicina, Santiago, Chile. (2) Universidad de Chile, International Center for Andean Studies (INCAS), Putre, Chile. Correspondence: eaherrera@uchile.cl

Background: Cardiovascular diseases (CVDs) are the leading cause of global mortality, and their incidence is increasing in the last decade1. Gestational hypoxia, which occurs in high altitude populations (≥2500m), is related to an increased risk to develop CVDs in postnatal life, including cerebrovascular problems2. In this study, we evaluate the effect of gestational hypoxia on the carotid morphology and arterial function in adult guinea pigs.

Methods: At gestational day 30, guinea pigs dams were introduced to a hypobaric chamber in normoxic (N, 720 torr, n=7) or hypoxic (H, 470 torr, n=7) conditions until term (~70 d). After delivery, individuals were euthanized at postnatal day 75 (adult) and carotid arteries were mounted in a wire myograph. Cumulative concentration-response curves (CCRC) to potassium chloride (KCl), phenylephrine (PE), endothelium-independent (Sodium nitroprusside-SNP) and -dependent (Methacholine-MetCh) were performed. Additionally, a histomorphological analysis was performed and all data were expressed as mean ± SEM and compared by Mann-Whitney test. Significant differences were accepted when $p \leq 0.05$.

Results: Hypoxia reduced the maximal K+-induced response and SNP sensitivity, whereas it increased the maximal PE-induced response when compared with the normoxic group (Figure1). The adventitial layer of the hypoxic artery had a larger surface area and a greater number of arteries (vasa vasorum) than the normoxic artery. The hypoxic middle layer was thinner and had a greater cell density than the normoxic layer.

Conclusions: Hypobaric hypoxia during gestation alters vascular responses and structure of carotid artery in the adult life. The maximal response induced by PE could be related to a higher number of nuclei as a sample of proliferation to compensate for a thinner medial layer relative to a normoxic artery. Further studies will assess the involved mechanisms and the persistence of these responses further in life.

Financing: Funding: Beca ANID 21191677, ANID 21230893, FONDECYT INICIO 11200798, FONDECYT Regular 1201283.

The imprinting of gestational hypothyroxinemia in the expression of defensins by Paneth cells

M. Jose Mendoza-León[1,2], Omar Vallejos[2,3], Oscar Álvarez-Mardonez[1,2], Maria A. Rangel-Ramírez[1,2,4], Enrique González-Madrid[1,2], Esteban Caamaño[1,2], Alejandro Regaldiz[1,2,5], M. Cecilia Opazo[2,5], Susan Bueno[2,3], Felipe Melo-González[1,2], Claudia A. Riedel[1,2]

(1) U. Andrés Bello, Fac. de Ciencias de la Vida, Santiago, Chile. (2) Millennium Institute on Immunology and Immunotherapy, Santiago, Chile. (3) Pontificia Universidad Católica de Chile, Fac. de Ciencias Biológicas, Santiago, Chile. (4) U. Andrés Bello, Fac. de Medicina y de Ciencias de la Vida, Santiago, Chile. (5) U. de las Américas, Fac. de Medicina Veterinaria y Agronomía, Santiago, Chile

Background. Maternal hypothyroxinemia (HTX) is a thyroid condition characterized by a reduction of maternal L-3,5,3',5'-tetraiodothyronine (T4) with normal levels of thyroid-stimulating hormone (TSH) and L-3,5,3'-triiodothyronine (T3), causing irreversible consequences to the central nervous system of the progeny. Given that this condition has been associated with an increased risk of developing autistic spectrum disorder (ASD) in the offspring. It is relevant to evaluate whether gestational HTX affects the gastrointestinal system as it has been observed in patients with ASD. Thus, in this work, Paneth cells (PC) from the ileum of the offspring gestated in HTX were analyzed given that this cell plays important roles in intestinal homeostasis, host defense, and immunity through the production of antimicrobial proteins and peptides (AMPs). Methods. C57BL/6 pregnant mice during embryonic days (E)10-E14 were treated with 0.02% methimazole in the tap-drinking water to induce gestational HTX. Control pregnant mice received tap-drinking water. The third experimental group received 0.02% MMI plus intraperitoneal administration of T4 to recover the effect of low T4 induced by MMI. As it was approved by bioethical committee of the Universidad Andrés Bello. The adult offspring at eight weeks old were euthanized, and the ileum was obtained for the evaluation of the mRNA content of AMPs like Lysozyme, Cryptidin-1, Cryptidin-4, and RegIIIγ were analyzed by RT-qPCR. Results: The male HTX-offspring showed increased expression of Lysozyme, Criptidin-1 and Criptidin-4 mRNA compared to the control offspring, suggesting an abnormal transcriptional profile. Conclusions: This work shows that PC granules from the HTX-offspring could have altered their antimicrobial peptide program expression, suggesting changes to the intestinal homeostasis and microbiota.

Keywords: Gestational Hypothyroxinemia, Antimicrobial Peptides, Paneth cells

Financing: AAEC 2, 2023-2024, UNAB. ANID DOCTORADO NACIONAL 21211419, 21202085, 21202356. Proyecto Núcleo UNAB DI-04-22/NUC; and FONDECYT #1191300 (CR), 11200764 (FM-G), ID20I10082 (SB), IMII ICN2021_045. Acknowledgments: Advanced Microscopy Facility UMA UC.

Correlation between maternal levels of polycyclic aromatic hydrocarbons and birth height in girls and boys.

Carolina Muñoz-Zamorano[1], Ivo Carrasco-Wong[3], Ady Giordano A[2], Anyelo Durán Mojica[3], Sebastián E Illanes[4,5,6], Jaime Gutierrez[3], Delia Indira Chiarello[3]

(1) Universidad San Sebastián (USS), PhD Program in Chronic Disease, Faculty of Medicine and Science, Santiago, Chile. (2) Pontificia Universidad Católica, Faculty of Chemistry and of Pharmacy, Santiago, Chile. (3) USS, School of Medical Technology, Faculty of Medicine and Science, Santiago, Chile. (4) Universidad de los Andes, Center for Biomedical Research and Innovation (CiiB), and (5) Department of Obstetrics and Gynecology, Faculty of Medicine, Santiago, Chile. (6) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background. Polycyclic aromatic hydrocarbons (PAHs), which are bound to fine particulate matter (PM2.5), are considered a type of endocrine disruptor. The Environmental Protection Agency has identified 16 high-priority PAHs, including benzo(k)fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene. Research has shown that PAHs can penetrate the placental barrier and have potentially negative effects on the developing fetus. Our aim was to explore the correlation of various plasma metabolites of PAH in maternal blood with anthropometry of boys and girls at birth. Methods. The investigation was designed to quantify plasma PAH concentrations in pregnant women at the time of delivery (term birth). Pregnant women participants in the study (50) were volunteers recruited from the patient population of the Universidad de los Andes Clinic in the city of Santiago, Chile. The concentration of each PAH was determined using a gas chromatograph–mass spectrometer (PerkinElmer Clarus 680) coupled with a mass spectrometry instrument. The statistical analyses were conducted using Graphpad Prism. Data were log-normal, Pearson correlation coefficients were used to examine the strength of the statistical relationship between maternal plasma PAH levels and newborn anthropometry. This study has been approved by the ethics committee of the Universidad de los Andes with the code CEC2022058. Results. Three maternal plasma metabolites of PAH, benzo(k)fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene, were negatively associated with newborn length, and all associations were more pronounced in girls than in boys ($p<0.01$). No correlation was observed with newborn BMI or weight. Conclusions. Prenatal PAH exposure was positively associated with length at birth, especially in girls. Further studies are needed to confirm causality and explore long-term health effects.

Keywords: Pregnancy, Polycyclic aromatic hydrocarbons, Exposome, Birth size

Financing: CONICYT, PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2019, Folio N°PAI77190060 to DC. Fondecyt Regular #1221362, Chile to JG. Acknowledgments: Patricia Valdebenito, midwife at the Universidad de los Andes Clinic.

Effect of Metformin on the Activity of Placental Glucosamine-6-Phosphate synthase and Offspring of Wistar Rats

Jorge Armando Ortiz Ramírez[1], Mayra Cuéllar Cruz[2], María Luisa Lazo de la Vega Monroy[1], Gloria Barbosa Sabanero[1], Everardo López Romero[2]

(1) University of Guanajuato, Department of Medical Sciences, Division of Health Sciences, León, Guanajuato, Mexico. (2) University of Guanajuato, Department of Biology, Division of Natural and Exact Sciences, Guanajuato, Mexico

Background. Gestational diabetes, influenced by nutrient availability, affects both maternal and fetal health. Metformin, a widely used drug during pregnancy to lower maternal hyperglycemia. Nutrient availability can impact the O-GlcNAcylation cycle, which regulates cellular functions. GlcN-6-P synthase, a potential placental nutrient sensor, plays a critical role in this cycle. The effects of Metformin on its activity, the O-GlcNAcylation cycle, and offspring health remain unexplored. Objective. To evaluate the activity of GlcN-6-P synthase in the placenta of Wistar rats at two gestational periods (13 or 18 days), treated or not with metformin, as well as the neonatal outcomes of the offspring (anthropometry). Materials and methods. When rats treated or not with metformin, reached 13 or 18 days of the gestational period, placental tissue was extracted, and GlcN-6-P synthase activity was determined using the modified Morgan-Elson method. Anthropometric measurements of the offspring were also performed. Statistical significance: $p< 0.05$. This project was approved by the Ethics Committee (CIBIUG-P49-2022). Results. In untreated rats, the activity of GlcN-6-P synthase increased with gestational age, 6.7 units and 12.9 units at 13 and 18 days respectively. However, in metformin-treated rats, activity was reduced to 4.8 and 8.2 units at 13 and 18 days, respectively ($p<0.001$). Placental efficiency in untreated rats was 81.6% at 13 days and 12.8% at 18 days, whereas in the metformin-treated rats, it rose to 89.2% and (48.6%;$p<0.001$) respectively. Offspring weight at 13 days varied (0.51g untreated and 0.32g treated), and there was a significant weight difference at 18 days (4.4g untreated vs. 2.3g treated; $p<0.001$). There were no significant differences in height between the groups. Conclusion. Activity of GlcN-6-P synthase increases throughout gestation; metformin treatment reduces it. Additionally, metformin treatment enhances placental efficiency and reduces fetal weight, suggesting an impact of the enzyme on placental metabolism and fetal programming.

Keywords: Placenta, Placenta, GlcN-6-P synthase, Metformin., Fetal Programming, O-GlcNcylation

Financing: IDEAGTO/CONV704672022UG and CIIC-UG 056/2023. Acknowledgments: To Magdalena Hernández-Herrera for her technical support in the experiments, Yolanda Alvarado and Maritza Almanza for their technical support, Pedro Galván for his support as the responsible party of vivarium.

Evaluating the Use of "Chile Crece Contigo" Online Platforms Among Pregnant Women in Chile.

Virla Palma[1], Andrea Rodriguez[1], Maricela Pino[2], Manuel Ortiz-Llorens[1], Jessica Moya[3], Jaime Gutierrez[4], Fabian Pardo[5,6], Delia Chiarello[4]

(1) School of Obstetrics, Faculty of Health Care Sciences, Universidad San Sebastián (USS), Santiago Chile. (2) School of Obstetrics and Childcare, Faculty of Health and Social Sciences, U. de las Américas, Santiago, Chile. (3) School of Nutrition and Dietetics and (4) School of Medical Technology, F. of Medicine and Science, USS, Santiago, Chile. (5) Biomed. Research Center and (6) School of Medicine, U. de Valparaíso, San Felipe, Chile.

Background: Prenatal education significantly affects maternal and fetal health. In the Chile Crece Contigo (CHCC) program, health professionals deliver prenatal education through face-to-face workshops, distributing educational material where the use of digital platforms, such as a web page and/or mobile application, is promoted. However, it is not known whether pregnant women visit these digital platforms, so we propose to evaluate the use of these platforms by Chilean pregnant women. Methods. These results are part of the results of the classic randomized clinical trial (RCT) called mami-educ (ClinicalTrials.gov Identifier: NCT05114174). An online validated survey was applied to 122 pregnant women in the first and second trimester attended at the Family Health Centers (CESFAM) of the communes of El Bosque, Conchalí and San Felipe, with prior signature of informed consent. It was analyzed by CHCC digital platforms statement of use (web page and/or mobile application) according to variables such as age, nationality, preconceptional and/or gestational pathologies, educational level. All analyses were performed using Stata 16.0 with a significance level (alpha) <0.10. The study was approved by the Scientific Ethics Committee of Universidad San Sebastian (protocol number: 07072020), the Aconcagua Healt Service (protocol number: 26/2021) and the South Metropolitan Health Service (protocol number: 07-27012022). Results. Only 20.4% of the participants stated that they had accessed the website or the Chile Crece Contigo application at least once. Of these, 60% were pregnant women with a higher level of education (technical/university). No statistically significant differences were observed in the use of digital tools according to age, nationality, occupational status, and prevalence of preconception and/or gestational pathologies. Conclusions. The use of digital platforms is associated with pregnant women with higher educational level. The challenge is to find strategies for dissemination or design of platforms aimed at pregnant women with lower levels of education.

Keywords. Primary care, mHealth, pregnancy.

Financing: Funding. Institute of Public Policies USS (IPSUSS1909); ANID-FONDEF, ANID-FONIS-7698SA21I0099.

Identifying facilitators and barriers influencing dietary decisions during pregnancy in 11 health care centers in Chile.

Fabián Pardo[1,2], Ayleen Bertini[1,3,4], Virla Palma[5], Andrea Rodriguez[5], Maricela Pino[6], Jessica Moya[7], Jaime Gutierrez[8], Delia Indira Chiarello[8]

(1) U. of Valparaiso (UV), Fac. of Medicine, San Felipe, Chile. (3) Programa PhD en Cs. e Ingeniería para la Salud, UV, Valparaíso, Chile. (4) Millennium Institute for Intelligent Healthcare Engineering, Valparaíso, Chile. (5) Universidad San Sebastian (USS), School of Obstetrics, Fac. Of Health Care Sciences, Santiago, Chile. (6) U. de las Américas, School of Obstetrics and Childcare, Fac. of Health and Social Sciences, Santiago, Chile. (7) School of Nutrition and Dietetics, Fac. of Health Care Sciences and (8) School of Medical Technology, Fac. of Medicine and Science, USS, Santiago, Chile.

Background. Gestational weight control is crucial for improving maternal and infant health outcomes. This study aims to identify facilitators and barriers influencing dietary decisions among pregnant women. Methods. The study was conducted as part of the mami-educ randomized clinical trial (ClinicalTrials.gov NCT05114174). An online survey was administered to 168 pregnant women from 11 CESFAMs in the Metropolitan and Valparaiso Regions, Chile. Study approved by the Scientific Ethics Committee of Universidad San Sebastian (Protocol #07072020), the Aconcagua Health Service (Protocol #26/2021), and the South Metropolitan Health Service (Protocol # 07-27012022). Results. Personal taste was identified as the primary barrier for increasing fruit (37.5%) and vegetable (35.7%) intake. Price was also a significant barrier (33.9% for fruits and 31.5% for vegetables). The primary barriers to water consumption were personal taste (54.2%) and awareness of positive/negative effects (28%). Regarding sugar consumption, personal taste (40.5%) and medical indications (39.9%) played crucial roles, while medical indications (40.5%) and personal taste (39.9%) were the main factors influencing salt consumption. Additionally, the study found that information about the risks of weight gain on the mother's health (93.1%) and the baby's health (95.4%) influenced pregnant women's decision to control their weight during pregnancy. Most participants expressed their willingness to stay within the recommended weight range if advised by their midwife (90.0%) or nutritionist (92.3%). Conclusions. While factors like price and personal taste influence pregnant women's dietary decisions, the majority emphasized the importance of knowing the risks of weight gain to facilitate gestational weight control. Targeted interventions and support strategies informed by these findings can promote healthier dietary behaviors among pregnant women.

Keywords: pregnancy, weight managment, facilitators, barriers

Financing: Institute of Public Policies of the San Sebastian University (IPSUSS1909); ANID + FONDEF/XVIII Concurso Nacional de Proyectos de Investigación y Desarrollo en Salud, FONIS 7698 (SA21I0099).

Characterization of pregnant women included on the randomized clinical trial: mami-educ

Fabián Pardo[1,2], Ayleen Bertini[1,3,4], Virla Palma[5], Andrea Rodriguez[5], Maricela Pino[6], Jessica Moya[7], Jaime Gutierrez[8], Delia Indira Chiarello[8]

(1) U. of Valparaiso (UV), Fac. of Medicine, San Felipe, Chile. (3) Programa PhD en Cs. e Ingeniería para la Salud, UV, Valparaíso, Chile. (4) Millennium Institute for Intelligent Healthcare Engineering, Valparaíso, Chile. (5) Universidad San Sebastian (USS), School of Obstetrics, Fac. Of Health Care Sciences, Santiago, Chile. (6) U. de las Américas, School of Obstetrics and Childcare, Fac. of Health and Social Sciences, Santiago, Chile. (7) School of Nutrition and Dietetics, Fac. of Health Care Sciences and (8) School of Medical Technology, Fac. of Medicine and Science, USS, Santiago, Chile.

Background. Correct gestational weight is crucial during pregnancy to avoid perinatal complications related to nutritional inadequacy. This study aims to intervene with nutritional education addressing cognitive, affective, and psychomotor domains among pregnant women attending family health centers (CESFAM). This report characterizes the volunteers included in this study. Methods. The study was conducted as part of the mami-educ randomized clinical trial (ClinicalTrials.gov NCT05114174), and pregnancy women's characteristics were obtained from 169 pregnant women from 11 CESFAMs in the Metropolitan and Valparaiso Regions, Chile. The study was approved by the Scientific Ethics Committee of USS (protocol #07072020), Aconcagua Health Service (protocol #26/2021), and South Metropolitan Health Service (protocol #07-27012022). Results. The mean age of women is 28.1±6.1 (18-44). The average gestational age at enrollment is 19.2 ± 8.0 weeks. Regarding previous pregnancies, 37.3% of the women have no previous pregnancies, 36.1% are in their 2nd pregnancy, and 26.6% have had ≥3 pregnancies. The BMI is 29 ± 5.6 kg/m^2 (1.8% underweight, 26% normal weight, 34.9% overweight, and 37.3% obese). Most participants (84%) are Chilean. Regarding education, 21.3% completed college, 39.6% completed high school, and 52.1% were employed. Most (78.7%) reported never having abnormal urine test results, and 96.4% were non-smokers. Regarding clinical appointments, 91.7% sees a midwife, and 61.1% visit a nutritionist. Conclusions. This study included a diverse group of women with different pregnancy histories, high prevalence of overweight and obesity, and predominantly Chilean. The participants generally reported a low smoking rate and attended midwives and nutritionists during pregnancy. These results provide information on the characteristics of the population studied and the patterns of use of health services.

Keywords: pregnancy, gestational obesity, characteristics, Chile

Financing: Institute of Public Policies of the San Sebastian University (IPSUSS1909); ANID + FONDEF/XVIII Concurso Nacional de Proyectos de Investigación y Desarrollo en Salud, FONIS 7698 (SA21I0099).

Gestational hypoxia elicits adverse cardiovascular phenotype in adult female guinea pigs.

Adolfo A Paz[1], Tamara A Jimenez[1], Jorge Mocarquer[1], Esteban G Figueroa[2], Felipe Beñaldo[1], Julieta Ibarra[1], Julieta Frey[1], Alejandro Gonzalez-Candia[2], Emilio A Herrera[1,3]

(1) ICBM, Faculty of Medicine, University of Chile, Santiago, Chile. (2) Fetal Neuroprogramming, Institute of Health Sciences, Universidad de O'Higgins, Rancagua, Chile. (3) International Center for Andean Studies (INCAS), Universidad de Chile, Putre, Chile

Background: Gestational hypoxia (GH) occurs in conditions such as placental dysfunction and high-altitude exposure, and it is associated with an increased risk of cardiovascular disease in adulthood1. GH may program cardiac dysfunction and remodeling, and a long-lasting imbalance of antioxidant and vasodilator systems. Nevertheless, most studies accounting for postnatal cardiovascular phenotype are overrepresented in male models. We aimed to determine the long-term effects of GH in cardiac structural, antioxidant and vasodilator characteristics on female guinea pigs, a recognized translational animal model. Methods: All procedures were approved by the local Ethical Committee (20354-MED-UCH). At gestational day 30 (term~70d), twelve pregnant guinea pigs were introduced in a hypobaric chamber in simulated-normoxia (720 torr, n=6) or hypoxia (470 torr, n=6) until term. After delivery, female newborns were maintained in normoxia. At one-year old, animals were euthanized, and hearts were processed for histology and molecular biology assays. Ventricle thickness and luminal area were assessed with Fiji software. In addition, fibrosis, and both antioxidant (NRF2) and inflammation (NFκB) main regulators were determined by Van Gieson stain or immunohistochemistry, and transcriptional levels of vasodilator enzymes (Nos3 and Hmox-1/2), antioxidant enzymes (Cat, Gpx1-4, and Sod1/2) and contractile protein (Myh7) were evaluated by qPCR. Data were analyzed by Mann-Whitney t test, and $p \leq 0.05$ was considered significant. Results: In one-year old female guinea pigs, body and heart weights unchanged among groups. Nevertheless, both left (LV) and right (RV) ventricles exhibited increased levels of fibrosis. In addition, RV structural parameters were similar between groups, but hypoxia increases NFκB expression and reduces Hmox-1 mRNA levels. In contrast, LV of the hypoxic group exhibits an increased luminal area, reduced Myh7 and increased Nos3 mRNA levels. Conclusions: These results indicate that GH induces a long-term cardiac effect in adult females, potentially leading to impaired cardiac function.

Keywords: Gestational hypoxia, Cardiovascular function, Cardiac Remodeling

Financing: Funding: FONDECYT 1201283, FONDECYT INICIO 11200798, ANID 21230893 and ANID 21191677.

Multiples or single gestation has differential effects on morphometry in testis adult Pelibuey rams

Tania Arellano[2], Helen Viotti[4], Anayansi Ramírez[3], **Graciela Pedrana[4]**, Graeme B Martin[1]

(1) University of Western Australia, Institute of Agriculture, School of Agriculture and Environment, Perth, Australia. (2) Universidad Autónoma Metropolitana-Unidad Iztapalapa, Biología de la Reproducción, Área de Sistemas de Producción Agropecuarios, Ciudad de México, México. (3) Colegio de Postgraduados, Campus Montecillo, México. (4) Universidad de la República, Facultad de Veterinaria, Montevideo, Uruguay

Background. Multiple fetuses are generally thought to cause intrauterine competition that affects fetal growth and development. Lambs born from multiple litter fetuses have intrauterine growth restriction caused by relatively small placentae with fewer cotyledons with lower birth weights1. However, previous studies describe a subsequent neonatal catch-up growth2. The link between low birth weight and lower fertility in adult males have been analyzed in single animals. So, we hypothesized that multiple fetuses would affect testis development in sheep. We compared morphometric measures of the testis in rams born as a single with those born as members of litters of two or three lambs. Methods. The protocol was approved by COBIAN-ColPos (Comité de Bienestar Animal del Colegio de Postgraduados: COBIAN/011/22). Testis samples from Pelibuey rams (n = 12) born as single-lamb litters (n = 4), twins and (n = 4) or triplets (n = 4) were analysed. Seminiferous tubule diameter (µm) were measured in transversal cross sections of seminiferous tubules and the number of Sertoli cells per ¼ cross section of seminiferous tubules (means± eem). Data were analyzed with ANOVA. Results: There were fewer Sertoli cells following single-fetus gestations (3.1 ± 0.13) than in multiple-fetus gestations (3.9 ± 0.1, 4.2 ± 0.1, P > 0.0001). Seminiferous tubule diameters were lower in single-fetus gestation (275.4 ± 4.1 µm) than multiple-fetus gestations (281.1 ± 4.1, 280.2 ± 4.1 µm, P > 0.0001). Conclusions: Intrauterine competition appears to reduce testis development, with potential effect on puberty and fertility in adulthood. Multiple births are considered attractive for flock productivity yet there may be disadvantages if these animals pass on these characteristics to their offspring. Clearly, genetic studies are needed in the Pelibuey breed.

References: 1.Gootwine et al. (2007). Animal. 1 (4): 547-564. 2. Horton et al. (2016).J Dev Orig Health Dis.7(5):548-562

Keywords: testis, gestation, ram, programming, Sertoli

Financing: Colegio de Postgraduados, Campus Montecillo, Maestría en Recursos Genéticos y Productividad-Ganadería

Maternal supplementation during the third trimester of gestation decreases Sertoli cell number and sex cord diameter in newborn lambs.

Paula Lombide[1], María Helen Viotti[1], Lilena Folgar[1], Leda Magariños[1], Beatriz Mernies[2], Graeme B Martin[4], Luis Cal[3], **Graciela Pedrana[1]**

(1) Biociencias, (2) Producción Animal, (3) Patobiología, Universidad de la República, Facultad de Veterinaria, Montevideo, Uruguay. (4) University of Western Australia, Institute of Agriculture, School of Agriculture and Environment, Perth, Australia

Background. Maternal nutrition during pregnancy influences the sperm production in adulthood1,2. In rams, the number of Sertoli cells determines the maximum number of spermatozoa that are produced3. In Merino sheep, maternal supplementation from 70 days of gestation until birth tends to increase the number of Sertoli cells in extensive conditions4. We compared two periods of maternal supplementation to test whether the effect on the newborn testis depends on the trimester of treatment. Methods. The experimental design was approved by the Committee on Ethics of Facultad de Veterinaria (CEUA N°635). Pregnant Corriedale ewes maintained on natural pastures were allocated in Control (no supplement; n = 10), Supplemented from 70 (Supp70; n = 10) or supplemented from 110 days of gestation until birth (Supp100; n = 10). At birth, Sertoli cells were counted, and sex cord diameter was measured. Data were analysed with ANOVA. Results. Sex cord diameter was smaller (P = 0.0001) in Supp110 (38.0 ± 0.3 µm) than in both the Control (40.6 ± 0.2 µm) and Supp70 groups (40.2 ± 0.3 µm). There were fewer (P=0.0001) Sertoli cells per sex cord transversal section in Supp110 (9.4 ± 0.2) than in the Control (11.6 ± 0.1) or Suppl70 (10.5 ± 0.2). Conclusions. Nutritional supplementation of ewes during the second trimester did not have affect sex cord diameter or Sertoli cell number in newborn lambs. By contrast, supplementation during the third trimester decreased both variables, and could lead to problems for sperm production in adulthood. Future studies will need to test whether these effects involve changes in transcription factors produced by Sertoli cells that program spermatogenesis in adult rams. References. (1.) Martin. (2022). Anim Reprod. 5;19(4):e20220088. (2.) O'Donnel et al. (2022). Semin. Cell Dev. Biol. 121: 2-9. (3.) Ferramosca & Zara. (2022). Int. J. Mol. Sci. 23(5): 2542. (4.) Bielli et al. (2001). Small Rumin Res. 40(1):63-71.

Keywords: Fetal-programming, ovine, testis, Sertoli cell, sex cords

Financing: Dedicación Total, Graciela Pedrana, Comisión Sectorial de Investigación Científica (CSIC), Comisión de Investigación y Desarrollo Científico (CIDEC) TR-697, Paula Lombide. Luis Cal proyecto CSIC, Universidad de la República, Uruguay.

Acknowledgments: The authors would like to thank Silvana Soria, Victoria Yozzi for laboratory procedures, Cecilia Abreu, Pablo Rodriguez, Karina Neimaur for lamb sampling.

Birth weight and insulin resistance plus dysglycaemia: a comparative study from the Intergenerational Limache Cohort

Isabel Pereyra[1], Sandra López-Arana[2], Josiemer Mattei[3]

(1) Universidad Católica del Maule, Facultad de Ciencias de la Salud, Curicó, Chile. (2) Universidad de Chile, Departamento de Nutrición, Facultad de Medicina, Santiago, Chile. (3) Harvard University, Department of Nutrition, Boston, United States

Birth weight, breastfeeding and early growth has emerged as underlying mechanisms explaining the increase onset of type 2 Diabetes Mellitus (DM2). Nonetheless, there is scarce information focusing on associations between these determinants operating during different nutrition and epidemiological transition periods. This study aimed to investigate the association of birth weight with insulin resistance (IR) plus dysglycaemia in Chilean young adults including a comparative analysis from two cohorts living in the same location at two different stages of nutrition and epidemiologic transition. A probabilistic sample of 1947 individuals (22-28 years) that belong to two birth cohorts (cohort 1: participants born in 1974-1978, cohort 2: participants born in 1988-1992). Birth weight was classified as low (< 2,500g), insufficient (2,500-3,000g), normal (3,000-4,000g) and macrosomic (> 4,000g). Suboptimal birth weight was considered when birth weight was low or insufficient. IR was defined by HOMA-IR ≥ 2.6 or by QUICKI < 0.33. Dysglycaemia was defined as fasting plasma glucose (FPG) ≥ 100 mg/dL. Multivariable logistic regressions were performed. The study was approved by the Ethic Committee of Faculty of Medicine of University of Chile, Number: 067/2013. For cohort 1, birth weight was negatively associated with IR plus dysglycaemia but this association was not statistically significant (adjusted odds ratio [AOR]: 0.78 (95% CI: 0.58-1.04). In cohort 2, IR plus dysglycemia was associated with birth weight; those born with more weight experienced a lower likelihood for IR plus dysglycemia (AOR:0.72; 95% CI: 0.54-0.96). When the analysis was done by suboptimal birth weight, in cohort 2 the association was significant (AOR: 1.40, 95% CI: 1.01-1.95). There was no association of macrosomia with IR and dysglycemia. Our findings suggest that being born in an epidemiological and nutritional transition and growing up during post-transition may be a possible mechanism for developing IR plus dysglycemia during adulthood when having lower birth weights.

Keywords: birth weight, Diabetes mellitus, insulin resistance, nutritional transition

Financing: The Intergenerational Limache Cohort was supported by Wellcome Trust and ANID-FONDECYT, Chile). This research was sponsored by ANII- Uruguay.

Acknowledgments: We acknowledge the study participants and the research team. We thank Prof. Patricia Bustos for her help with data interpretation and the deceased Prof. Hugo Amigo, who provided general support.

Maternal high fat diet preconception changing to normolipidic during pregnancy and lactation and offspring metabolism

Esther Alves de Souza, Laís Vales Mennitti, Yasmin Alaby Martins Ferreira, André Wu Chen, Marcela Nascimento Sertório, Andrea Jucá, **Luciana Pellegrini Pisani**

Federall University of São Paulo, Biosciences, Institute of Health and Society, Silva Jardim 136, Vila Mathias, Santos, Brazil

Background: Preconceptional, gestation and lactation are relevant to offspring health. Maternal diet is an important factor related to Developmental Origen of Health and Disease[1]. The high fat (HF) or normolipidic diet based on fatty acids quality inadequacy could lead the decedents to metabolic and epigenetic alterations[2],[3]. Maternal intake of polyunsaturated fatty acids could be an approach to reverse the effect of maternal HF diet before conception. Also, the metabolism can respond differently considering the sex-dependent. Methods: We evaluated in the female progenitor: oral glucose tolerance test (OGTT) and area under the curve (AUC) and serum insulin by ELISA. In male and female offspring: carcass protein and lipid content; protein expression of glucose metabolism and epigenetic markers in the hypothalamus and visceral adiposity. All procedures were approved by the ethics committee (CEUA n. 7330131021). Results: The OGTT and AUC showed glucose intolerance in HF group preconception. In the offspring, the content of lipid and protein in the carcass were similar among groups. However, the sum of visceral adipose tissue was higher in HF-HF21 male and female, compared to the others. Also, HF-HF21, HF-C21 and HF-S21 female were lower compared to their respective male groups. We found no differences in the expression of insulin receptor (IR), insulin receptor substrate-1, DNMT3a, HDAC11, Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α and sirtuin 1 (SIRT1), although PGC-1 was higher expressed in female HF-C21 and SIRT1 higher expressed in female HF-S21 than in their male respective groups. Conclusion: Maternal HF diet before conception could lead female progenitors to glucose intolerance. This seems to affect offspring metabolism even though in pregnancy and lactation has changed to a normolipidic diet, leading to higher adiposity in HF-HF21 offspring. Also, we could see the sex dimorphism in visceral adiposity and expression of proteins involved in the cell metabolism.

Keywords: maternal high-fat diet, offspring, sex dimorphism

Financing: The authors gratefully acknowledge São Paulo Research Foundation (FAPESP #2021/10375-8 and #2022/05529-9) and CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (#305725/2020-3).

Analysis of metabolic parameters in the adult offspring gestated in hypothyroxinemia and fed a cafeteria diet

Alejandro Regaldiz[1,2,3], Johana Santos[2,3], Nicole Avilés[2,3], M. José Mendoza-Leon[2,3], Ma. A. Rangel-Ramírez[2,3], Enrique Gonzalez- Madrid[2,3], Oscar Álvarez-Mardonez[2,3], Esteban Caamaño[2,3], Claudia A. Riedel[2,3], Ma. Cecilia Opazo[1,2]

(1) Instituto de Ciencias Naturales, Facultad de Medicina Veterinaria y Agronomía, Av. Chile. (2) Millennium Institute for Immunology and Immunotherapy, Santiago, Chile. (3Universidad Andrés Bello, Facultad de Ciencias de la Vida, Santiago, Chile

Background: The rapid global upsurge in obesity and type 2 diabetes (T2D) cannot be solely attributed to the consumption of a Western diet. It has been reported that exposure to adverse conditions during fetal development influences metabolic programming, thereby increasing the risk of offspring developing metabolic disorders later in life. For instance, maternal hypothyroxinemia (HTX), characterized by maternal thyroxine (T4) deficiency, has been associated with detrimental effects on offspring in both human and animal models. Nonetheless, the interplay between gestational exposure to HTX and later-life consumption of a Western-like diet on metabolic function remains a subject of inquiry. Methods: Male C57BL/6 mice, gestated or not in HTX, were fed either a cafeteria (CAF) or standard (ND) diet for ten weeks (n = 8-10 animals/group). Metabolic parameters (triglycerides, cholesterol, glucose tolerance, and insulin tolerance) were assessed before and after dietary intervention. Additionally, continuous monitoring of food intake and body weight was conducted throughout the experimental period. Furthermore, transcripts for glucose transporters GLUT1 and GLUT4 were measured by RT-qPCR on white adipose tissue. Statistical differences were evaluated using the Mann-Whitney test for two-group comparisons and Two-Way ANOVA for multiple comparisons. The Bioethics Committee at Universidad Andres Bello reviewed and endorsed all animal and experimental procedures. Results: Prior to dietary intervention, HTX-offspring exhibited increased glucose and insulin intolerance compared to the control group. Additionally, HTX-offspring showed greater weight gain relative to CTRL-offspring during the dietary intervention, with differences emerging sooner in the CAF-fed subgroup. Both HTX- and CTRL-offspring fed a CAF diet demonstrated elevated glucose intolerance compared to their ND-fed counterparts. Notably, HTX-offspring-fed ND and CAF diets experienced elevated triglyceride levels. Conclusions: Gestational exposure to HTX contributes to the development of metabolic-related alterations in mice. This study underscores the pivotal role of early-life factors in shaping metabolic health.

Keywords: metabolic programming, maternal hypothyroxinemia, cafeteria diet

Financing: UNAB DI-02-21/APPUDLA DI-08/22UNAB DI-02-23/APPPROGRAMA ICM - ANID, ICN09_016.

Exploring Barriers and Facilitators to Surgical Referrals for Neonates with Congenital Anomalies.

Natasha Ross[1], Elizabeth Alvarez[2]

(1) McMaster University, Global Health, Health Sciences, Hamilton, Canada. (2) McMaster University, Health Research Methods, Evidence and Impact, Health Sciences, Hamilton, Canada

Background. Advancements in medicine have resulted in decreased neonatal mortality and morbidity associated with congenital anomalies (CA). Unfortunately, the advantages of these developments have been confined to high-income countries (HICs), demonstrated by the comparatively high incidence of congenital anomalies in low and low-middle-income countries (LLMICs). Evidence suggests that neonates in LLMICs encounter considerably more barriers to care than those in HICs due to a malfunctioning referral system and poorly implemented health policies that hinder the timely provision of care.1,2 As many CA are now accepted as surgically treatable, the purpose of this study was to understand what inhibits the success of a neonate from obtaining surgery in LLMICs and how that could be improved.

Methods. Seven databases were searched in this systematic review to identify articles on neonates with surgically treatable CA. A total of 370 studies were screened; 16 were included in the final analysis. Studies were screened and selected individually by two researchers and all disagreements were resolved jointly. Studies were reviewed for factors affecting the delivery of surgical treatment and were then coded as a barrier or a facilitator.

Results. Active barriers to care were identified in every study, and facilitators were identified but were not active in impacting the acquisition of surgery. This study provides additional detail on what is known about the surgical referral system in LLMICs.

Conclusions. The study findings will inform policymakers of the realities faced by neonates and their caregivers while navigating through the surgical referral system and establish the need for alternate policy implementation strategies.

Keywords: Congenital anomalies, Neonates, Health equity, Surgery, LMIC

Financing: No funding.

Gestational Chronodisruption and its Impact on Inflammaging: Differential Metabolic and Inflammatory Profiles in Male and Female Offspring.

Katiushka Sanhueza[1], Natalia Mendez[1], Fernando Corvalan[1], Diego Halabi[1], Abigail Vasquez[1], Pamela Ehrenfeld[1], Karina Vergara[1], Claudia Torres-Farfan[1]

(1) Universidad Austral de Chile, Instituto de Anatomía, Histología y Patología, Medicina, Edificio Ciencias Biomedicas, Isla Teja SN, Valdivia, Chile

Background: Inflammaging is linked to non-communicable diseases (NCDs) and elevated mortality. Circadian disruptions, as seen with shift-work or photoperiod changes, heighten proinflammatory cytokines, hinting at NCDs. Evidence indicates that gestational chronodisruption can set offspring on a path to inflammaging, with White Adipose Tissue (WAT) central to this trajectory(1–4). Our study assesses maternal chronodisruption's influence on offspring's inflammatory and metabolic profiles.Methods: Female rats maintained in photoperiod 12:12 light:dark were mated and separated into A) control photoperiod 12:12 (LD; n=8); B) chronic phase shift photoperiod (CPS; n=8); and C) CPS mothers supplemented with melatonin in the drinking water during subjective night (CPS+Mel; n=8). At 18 days of gestational age, pregnant dams returned to photoperiod 12:12. In the male and female offspring (from 90 to 400 days old), we evaluated the weight gain, adipose tissue composition, glucose tolerance test, and in plasma AM/PM plasma levels of cytokines, corticosterone, and melatonin. Protocols were approved by IACUC-UACh #CBA-352. Results: CPS Male offspring display increased body weight, higher than control and melatonin-treated groups. At 200 days, their total fat content was also higher with an elevated glucose response to fasting and intraperitoneal glucose challenges across their lifespan, peaking in the Area Under the Curve of the glucose challenge by 400 days. Females remained unaffected in this regard. As well, male and female CPS offspring showed diminished day/night variations in melatonin and corticosterone levels, with irregular pro-inflammatory cytokine patterns for IL1a, IL6, and IL10. Maternal melatonin treatment reversed the effects on male metabolism and on female and male inflammaging. Conclusions: Our findings underscore that inflammatory alterations in males precede the metabolic impact of chronodisruption, with significant inflammatory markers appearing by day 90. This suggests that gestational chronodisruption may program low-grade inflammation in utero. While both sexes manifested inflammatory responses, only males display metabolic disorders.

Keywords: Inflammaging, Obesity, Circadian Rhythms

Financing: Fondecyt 1191207

Acknowledgments: Escuela de Graduados, Facultad de Medicina, Universidad Austral de Chile.

Effect of White Strawberry Polyphenols supplementation on metabolic parameters of the offspring from maternal obesity

Viviana Valero[1], Samanta Thomas[2], Gonzalo Jorquera[3]

(1) Programa de Doctorado en Ciencias e Ingeniería para la Salud, Facultad de Medicina, Universidad de Valparaíso, Valparaíso, Chile. (2) Escuela de Nutrición y Dietética, Facultad de Farmacia, Universidad de Valparaíso, Valparaiso, Chile. (3) Centro de Neurobiología y Fisiopatología Integrativa (CENFI), Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso, Valparaiso, Chile

Background. The offspring of obese mothers are affected by inflammatory and metabolic alterations1. Several of those dysfunctions are caused by changes in the expression of SIRT1 and downstream of enzymes related to lipid metabolism2. Polyphenols exhibit antioxidant and anti-inflammatory activities that could improve these alterations in the offspring3. Our aim is to evaluate the effect of maternal consumption of a polyphenolic extract of Chilean white strawberry during pregnancy and lactation on expression of lipid metabolism markers in the liver of the obese mother offspring at PND1 and 21. Methods. Approval of the Bioethics Committee Facultad de Ciecias UV CBC 07/2021. Sprague Dawley female rats were fed with high fat (HF) diet for 4 weeks prior to crossbreeding. Once the pregnancy was confirmed, they were divided into 4 groups: control (CT), CT + extract (CT+E), HF, HF + E. The extract was orally administrated to the mothers (200 mg/kg) during pregnancy and lactation. The expression of hepatic FAS, ACC, PCK and SIRT1 was determined by Western-Blot. The results are expressed as mean ± SD ($p<0.05$). Shapiro-Wilk test was used to determine the normality of the data, followed by OneWay ANOVA with Tukey's post-test or Kruskal-Wallis with Dunn's post-test. Results. CT+E pups showed higher body and liver weight at birth. A greater body, liver and adipose tissue weight was observed in HF vs CT pups. Newborn HF+E males have smaller liver size vs HF, while they showed more epididymal fat at PND21. There was lower expression of PCK2 and ACC in HF vs CT for both genders of pups. The extract increased expression of FASN in males. There was lower expression of PCK2 and ACC in HF vs CT in male pups, while ACC expression did not show difference. Conclusions. Polyphenolic supplementation during pregnancy and lactation could impact the metabolism of the offspring.

Keywords: Gestational Obesity, polyphenols, lipid metabolism

Financing: Initiation FONDECYT into research 11190762 (ST); Regular FONDECYT regular 1201816 (GC). Acknowledgments: FONDECYT (ST & GC), PhD Ingeniería y Ciencias para la Salud, Universidad de Valparaíso, Ramón Sotomayor (OmegaLUM). Laboratorio Alteraciones Reproductivas y Metabólicas, Farmacopea Chilena, Facultad de Farmacia technical staff.

Maternal resveratrol supplementation prior and during pregnancy prevents intrauterine growth restriction in rat maternal obesity

Lilia Vargas-Hernández[1,2], Carlos Alberto Ibáñez Chávez[1], Luis A Reyes-Castro[1], Claudia J Bautista-Carbajal[1], Elena Zambrano-González[1]

(1) Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Departamento de Biología de la Reproducción, Vasco de Quiroga 15, Belisario Domínguez Secc XVI, Tlalpan, 14080, Ciudad de México, México. (2) Instituo Mexicano del Seguro Social, Hospital de Ginecología y Obstetricia No.4 Luis Castelazo Ayala, Ciudad de México, México

Background. Maternal obesity significantly impacts offspring development, having multidirectional effects on fetal growth, such as intrauterine growth restriction (IUGR) and macrosomia. Fetal growth is related to maternal adiposity and gestational weight gain (1). Resveratrol supplementation has been proposed to counterbalance the effects of excessive maternal adiposity in experimental rats (2). We aimed to determine the effects of resveratrol supplementation in obese mothers on gestational weight gain and fetal growth as a function of maternal adiposity. Methods. Female Wistar rats (F0) were weaned to chow (C) or a high-fat diet (MO). One month before mating until 19 days' gestation (dG), half the F0 received 20 mg resveratrol/kg/d orally (CRes and MORes). All F0 were weighed during pregnancy and euthanized at 19 dG for visceral fat dissection and weighing. Immediately the fetuses were quickly euthanized and weighed. Gestational weight increase and maternal total fat at 19 dG were analyzed by 2W-ANOVA and proportions of Small for Gestational Age (SGA≥5th percentile of C fetal weight distribution) by $\chi 2$-test. Data are shown as mean±SEM and statistical significance at $P < 0.05$ (*). Procedures approved by INCMNSZ Animal Experimentation Ethics Committee. Results. Gestational weight increase until 19 dG (C=118±5g, CRes=104±6g, MO=111±7g and MORes=88 ±7g*vs. MO) was higher in MO than MORes. Maternal total fat (C=15±2g, CRes=12±2g MO=51±7*vs. C, MO=54±5g and MORes=51 ±7g*vs. MO) and SGA fetuses at 19 dG were increased in MO and MORes but decreased in CRes vs C (Fig. 1A). Fetal weight exhibited a significative U-shaped relationship with maternal weight (Fig.1B) and a stronger negative-linear relationship with the maternal total adipose tissue (Fig. 1C). Conclusions. Maternal high-fat diet before pregnancy led to IUGR. Resveratrol supplementation one month before and during pregnancy in obese mothers partially prevents IUGR. The resveratrol intervention reduced the gestational weight gain in obese mothers probably by modulating adipose tissue accumulation.

Keywords: Maternal Obesity, Fetal growth, Intrauterine growth restriction

Financing: Newton Fund RCUK-CONACyT (Research Councils UK—Consejo Nacional de Ciencia y Tecnología). I000/726/2016 FONCICYT/49/2016

Maternal high fat diet preconception and normolipidic based on different fatty acids effects on offspring

Esther Alves de Souza[1], Laís Vales Mennitti[1], Marcela Sertório[1], Aline Boveto Santamarina[1], Yasmin Alaby Martins Ferreira[1], Brendha Silva Andrade[1], Andrea Jucá[1], Luciana Pellegrini Pisani[1]

(1) Federal University of São Paulo, Biosciences, Institute of Health and Society, Silva Jardim, 136, Vila Mathias, Santos, Brazil

Background: Pregnancy and lactation periods are crucial in fetal and neonatal development[1]. Maternal diet during this period directly influences the development and health of offspring with different responses between males and females. The maternal high-fat diet (HF) can cause resistance to hormone action possibly associated with an inflammatory process, affecting lipid and glucose metabolism, intestinal permeability, and the hypothalamic function of the offspring. The maternal diet based on saturated fatty acids (SFA) may negatively affect fetal development and metabolism. In contrast, the maternal diet based on PUFA-n3 can improve offspring parameters impaired by the maternal HF diet based on SFA[2],[3]. Objectives: This work aims to evaluate the effect of a maternal high-fat diet in preconception and normolipidic diet with different types of fatty acids during pregnancy and lactation regarding inflammatory and energy regulation parameters of male and female offspring at 21 days of life. Methods: We evaluated in male and female offspring: inflammatory cytokines in the hypothalamus; leptin, lipopolysaccharides (LPS), neuropeptide Y (NPY), and glucagon-like peptide 1 (GLP-1) serum concentrations by ELISA. All procedures were approved by the ethics committee (CEUA n. 7330131021). Results: Maternal food intake (g) were lower in HF-HF in the 1st week (vs HF-C, HF-S, HF-P, $p<0.05$), 2nd week (vs HF-S, $p<0.01$), and 3rd week (vs all groups, $p<0.05$). In offspring, in the hypothalamus, the inflammatory cytokine IL-1β was lower ($p<0.001$) in the female HF-HF compared to male HF-HF. The serum analyses showed no difference in the leptin, LPS, or NPY, however, GLP-1 was lower in HF-HF male group than C-C and HF-P ($p<0.001$), and HF-HF female group ($p<0.01$). Conclusion: In the initial life developmental stage, the type of fatty acids – can influence and contribute to a system disorder in the offspring, mainly those descendent of those fed with HF before and during pregnancy and lactation.

Keywords: maternal diet, offspring hypothalamus, fatty acids

Financing: The authors gratefully acknowledge São Paulo Research Foundation (FAPESP #2021/10375-8 and #2022/05529-9) and CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (#305725/2020-3).

Embryo Development

Effects of fetal vitamin E deficiency and maternal gestational stress on early brain development in mice

Fujiko Saavedra[1], Nicolas Santander [2], Maxs Mendez[3], Trinidad Orellana[4], Luis Federico Batiz[3,5], Dolores Busso[1,5.]

(1) Program in Biology of Reproduction, Biomedical Research and Innovation Center (CIIB), UAndes, Santiago, Chile. (2) Instituto de Ciencias de la Salud, Universidad de O´Higgins, Rancagua, Chile. (3) Program in Neuroscience, CIIB, UAndes, Santiago, Chile. (4) Medical Student, Faculty of Medicine, UAndes, Santiago, Chile. (5) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, UAndes, Santiago, Chile.

Background. Fetal programming refers to how environmental stimuli during pregnancy affect the offspring´s development. Maternal stress and vitamin E (VitE) deficiency can increase fetal brain oxidative stress causing neurodevelopmental disruptions. The SR-B1 receptor is expressed in the placenta and takes up lipids (ie.VitE) from maternal lipoproteins. We compared VitE levels, lipoperoxidation, and morphometry of brains from SR-B1-/- and WT fetuses from stressed or control dams. Methods. After heterozygous intercrosses, dams were subjected (or not) to chronic social stress from E1.5-E16.5. Open field tests were performed to evaluate maternal stress. VitE content (HPLC) and lipoperoxidation (TBARS) were evaluated in brains, placenta, and plasma from WT and SR-B1-/- fetuses at E16.5. Fetal brain coronal sections were stained with H&E and subjected to morphometric analyses. Protocols were approved by the Ethics Committee from Universidad de los Andes (ID CEC2022113). Results. Stressed dams spent more time in the border of the open field (84.6% vs. the center, $p<0.0001$, n=7) compared to control dams (62.7 vs. 37.2% of the time in the center, p=0.19, n=3). Fetuses from stressed dams (n=118) had decreased fetal weight (0.47 vs. 0.51 g, p=0.0003), and decreased fetal/placenta ratio (5.7 vs. 6.2, p=0.0058) vs fetuses from controls (n=106). No significant differences were observed in morphometric comparisons in fetal brains from both groups. However, in the control group lower levels of VitE were observed in SR-B1-/- fetal brains (305 vs. 1074 pg/µg protein in WT, p=0.021, n=8) and livers (27.2 vs. 121.1 pg/µg protein in WT, p=0.06, n=7). SR-B1-/- fetal brains from control dams also showed increased lipoperoxidation (25.7 vs 18.3 nmol MDA/mg protein in WT, p=0.04, n=7). We are currently analyzing samples from the stressed group. Conclusions. Gestational stress affects fetal weight and SR-B1 expression may be necessary to maintain normal VitE levels and prevent lipoperoxidative damage in fetal brains.

Funding: FONDECYT#1221376 (to DB), and #1211384 (to FB) . ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024 (to DB and FB). Ph.D. fellowship ANID #21201204 (to FS).

Embryonic Origins of Cardiac Dysfunction in Developmental Hypoxia: The Role of miR-2-5p

Germán A. Arenas[1], Ranya Kumar[2], Youguo Niu[2], Sage Ford[2], Rachael Crew[2], Anna Cochrane[2], Dino A Giussani[2], Bernardo J Krause[3]

(1) Universidad Bernardo O'Higgins, Instituto de Ciencias de la Ingeniería, Rancagua, Chile. (2) University of Cambridge, Department of Physiology, Development and Neuroscience, Cambridge, UK. (3) Universidad de O'Higgins, Instituto de Cs. de la Salud, Rancagua, Chile

Introduction: Chronic fetal hypoxia is commonly observed in high-risk pregnancies, for example, in fetal growth restriction and preeclampsia, and it triggers a prenatal origin of heart disease (Giussani DA, 2021). Growing evidence suggests that epigenetic mechanisms may mediate the effects of developmental hypoxia (Ducsay CA et al., 2018). Nonetheless, the specific role of microRNAs in fetal cardiac dysfunction during hypoxic development remains unknown. Using the chicken embryo model to isolate the direct effects of treatment on the embryo independent of effects on the mother and/or placenta, we aimed to elucidate the role of miR-21-5p in developmental hypoxia-induced cardiac dysfunction. Methods: Fertilised Bovans Brown eggs were incubated under normoxia (21% O2) or chronic hypoxia (14% O2) from day 1 (term is 21 days). Chicken embryos were treated topically onto the chorioallantoic membrane with miR-21-5p Agomir (1µg/embryo/day) or vehicle (100 µL saline) on days 13, 15 and 17 of incubation. On day 19, embryos were euthanized via cervical transection and hearts were mounted on a Langendorff preparation to assess basal and stimulated cardiac function. This research was approved under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following an ethical review by the University of Cambridge Animal Welfare and Ethical Review Board. Results: Relative to controls, hypoxic embryos showed asymmetric growth restriction, diastolic dysfunction, sympathetic hyper-reactivity, enhanced basal Coronary flow rate (CFR) and an impaired CFR response to adenosine. Treatment of hypoxic embryos with Agomir miR-21-5p restored all effects and enhanced systolic function. Treatment of control embryos with Agomir miR-21-5p had no effects.Conclusions: Since miR-21-5p sequence is highly conserved between chickens and humans, Agomir miR-21-5p appears as a promising candidate for an improved human clinical translation. This approach could provide a valuable means of protecting offspring from the adverse effects of early onset heart disease associated with complicated pregnancies.

Keywords: Embryonic Origins - Hypoxia - Cardiac Dysfunction - microRNA

Financing: Funding: Supported by FONDECYT 1220421 and DoHAD Training Mobility Award 2021, the Lister Institute of Preventive Medicine, the British Heart Foundation, the Medical Research Councill, and Trinity College Cambridge.

Role of Vitamin E and Lipids in Neural Tube Defects: Insights from SR-B1 KO Embryos.

Gabriela Belledonne[1,2], Alonso Quiroz[3], Fujiko Saavedra[4], Dolores Busso[1,5]

(1) Universidad de los Andes, Biomedical Research and Innovation Center, Biology of Reproduction Lab, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, School of Medicine, PhD Program in Medical Sciences, Santiago, Chile. (3) Instituto de Investigación Materno Infantil, Santiago, Chile. (4) Universidad de los Andes, School of Medicine, PhD Program in Biomedicine, Santiago, Chile. (5) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background. Neural tube defects (NTD) are congenital malformations characterized by abnormal brain and spine formation caused by genetic and environmental factors, including malnutrition. Scavenger Receptor Class B Type 1 (SR-B1), expressed in early extra-embryonic tissues, transfers lipids (e.g. vitamin E) between HDL and cells. SR-B1-/- mouse embryos develop NTD with a 1:2 incidence and are vitamin E deficient. Maternal vitamin E supplementation completely prevents NTD in SR-B1-/- mice. It has been proposed that vitamin E may act as a liposoluble antioxidant protecting polyunsaturated fatty acids (PUFAs) from oxidation during neurulation. Methods. Lipoperoxidation was evaluated by malondialdehyde (MDA) levels (TBARs assay) and compared in SR-B1+/+ (WT) and SR-B1-/- E9.5 embryos from chow-fed and vitamin-E-supplemented-fed dams (n=5/group). The relative abundance of diverse species of PUFAs in phospholipids was compared among WT embryos and SR-B1-/- embryos without (nKO) or with NTD (NTD-KO) (n=3/group), using shotgun-lipidomics. Results. SR-B1-/- embryos showed higher MDA levels than WT ($p=0.02$), but the maternal diet did not affect this parameter (Two-way-ANOVA). The total levels of most highly unsaturated PUFAs were similar among WT, nKO and NTD-KO; however, the abundance of some saturated and monounsaturated fatty acids differed among groups. Interestingly, both KO groups showed less phosphatidylcholine (PC)/LysoPC ratio vs. WT ($p<0.05$/ANOVA). In NTD-KOs, less PC was observed vs. both morphologically normal groups ($p<0.05$/ANOVA). Conclusions. Our results suggest that the preventive effect of vitamin E on NTD in SR-B1-/- embryos is not mediated by preventing PUFAs lipoperoxidation. Lipidomic data suggest that vitamin E deficiency in SR-B1-/- embryos affects the composition of phospholipids. The production of LysoPC from plasma membrane PC is observed in various pathophysiological conditions, i.e. inflammation and endothelial activation. Thus, it may be hypothesized that vitamin E prevents the generation of lipid inflammatory mediators and protects neuroepithelial cells during neural tube closure.

Financing: Funding. FONDECYT #1180347 and #1221376 and ANID—Basal funding for Scientific and Technological Center of Excellence IMPACT #FB210024 (D.B), PhD-fellowship CONICYT #21170306 (A.Q), PhD-fellowship ANID #21222263 (G.B), PhD-fellowship ANID #21201204 (F.S).

Cisplatin-induced mitochondrial dysfunction alters cell cycle dynamics and differentiation in neural stem and progenitor cells

Felipe A. Bustamante-Barrientos[1,2], Noymar Luque-Campos[1,2], Patricia Luz-Crawford[1,2,3]

(1) Immunology Program, Center for Biomedical Research and Innovation (CIIB), Medicine Faculty, Universidad de los Andes, Santiago, Chile. (2) IMPACT, Center for Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile. (3) School of Medicine, Medicine Faculty, Universidad de los Andes, Santiago, Chile

Introduction. Long-standing evidence highlights the importance of mitochondria in the biology of stem cells. Neural stem and progenitor cells (NSPC) carrying mitochondrial dysfunction express a progressive decline in their ability to self-renew and differentiate toward neural cell types. Most pharmacologic methods aimed to induce mitochondrial dysfunction lead to the suppression or exacerbation of mitochondrial ATP production. Nevertheless, most of these approaches provoke short-time consequences, making it difficult to evaluate the impact of mitochondrial dysfunction in cellular processes like proliferation and differentiation. Methods. We underline the effectiveness of cisplatin, a broad-scope, and FDA-approved chemotherapeutic agent, as a pharmacologic approach to generate acute mitochondrial dysfunction in primary cultures of NSPC, which were directly obtained from the telencephalic tissue of mouse neonates. Colorimetric, flow cytometry, chemical analytic methods, and confocal microscopy were employed to evaluate mitochondrial functionality, cell cycle progression, and differentiation in NSPCs. Procedures were approved by the Bioethical Committee of the Universidad de los Andes following the NIH Guide for Care and Use of Lab Animals. Results. When used at doses below 10 uM, cisplatin maintains the NSPCC´s survival over 90%. Cisplatin IC50 (5 uM) induces mitochondrial dysfunction through membrane polarity, overall oxide-reductase activity, generation of reactive oxygen species, overexpression of mitochondrial fission markers, a metabolic switch towards glycolysis, and poor ATP content. Furthermore, cisplatin 5 uM increases NSPC´s cell cycle arrest and generation of neuronal progenitors/immature neurons and astrocytes under culture-specific conditions, while findings using 2.5 uM cisplatin suggest that changes in S- phase and M-G2 stages should be tested earlier than 24 hours. Conclusions. Our results support that cisplatin-induced mitochondrial dysfunction generates a bi-phasic response characterized by acute mitochondrial overactivity but significantly declines after 48 hours in vitro. It correlates with long-term modification in their cell cycle dynamic while their lineage-specific commitment toward neurons and astrocytes increases.

Keywords: Neurogenesis, Gliogenesis, Brain development

Financing: FONDECYT Postdoctorado 3220204

Mitochondrial E3 ubiquitin ligase MUL1 deficiency in mice causes impaired growth.

Ximena Calle[1,2], Brenda Becerra Leiva[2], Angelica Ortega[2], Valeria Garrido[2,3], Mayarling Troncoso Magnin[2], Claudia Muñoz[2], Bernardo Krause[1], Sergio Lavandero[2,3]

(1) University of O'Higgins, Rancagua, Chile, Institute of Health Sciences, Gene-environment interaction laboratory, Rancagua, Chile. (2) University of Chile, Santiago, Chile, Advanced Center for Chronic Diseases (ACCDiS), Faculty of Chemical; Pharmaceutical Sciences, Faculty of Medicine, Santiago, Chile. (3) Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas.

Background: MUL1 is a mitochondrial outer membrane protein with E3-ligase activity that regulates proteins involved in mitochondrial dynamics, which controls mitochondrial morphology and function. In addition, MUL1 inhibits cell growth, induces apoptosis, and modulates the immune system (1). Studies have reported that perinatally acquired epigenetic and microstructural alterations in metabolic and body weight regulatory systems appear critical because they lead to a cardiometabolic risk disposition throughout life (2). However, the effects of partial or total MUL1 deficiency in a whole organism are still unknown. This study aims to describe the effect of impaired MUL1 expression on development and growth trajectory in mice.

Methods: C57BL6N/ HET (n = 5), WT (n = 5), and KO (n = 3) MUL1 mice (CICUA approved protocol, 20351-CYQ-UCH) were genotyped by conventional PCR with specific primers at 21 days after birth, and the following parameters were assessed at 12 weeks of age: body weight and size, and cardiac hypertrophy markers. In addition, survival analysis (Kaplan-Meyer) was performed. Data were analyzed by nonparametric t-test and one-way ANOVA.

Results: The percentage of effective crossbreeding among the different genotypes was KO-KO 0%, HET-HET 64%, and KO-HET 75%. Therefore, KO animals in this study came from HET-HET and KO-HE breeds. Kaplan-Meyer analysis showed reduced survival of KO C57BL6N animals compared to HET and WT animals. Body weight and size were decreased in KO mice compared to HET and wild-type WT mice. Heart size normalized by mouse tibia length found no significant differences between WT, HET, and KO groups. However, differences in cardiac hypertrophy markers were observed in KO mice.

Conclusion: Total MUL1 deficiency in mice negatively influences fertility, development, and survival. Further studies will be conducted to identify molecular tissue-specific pathways programmed by impaired regulation of mitochondrial dynamics.

Keywords: MUL1, deficiency growth., Knockout

Financing: This work was funded by FONDAP 15130011 (SL), FONDECYT 1200490 (SL) and Postdoctoral UOH (XC) projects.

Comparison of the effect of piezoelectric and conventional microinjection on bovine embryo development and quality

Fernanda Fuentes[1,2], Erwin Muñoz[1,2], María Elena Arias[1,3], Luis Aguila[1], Ricardo Felmer[1,4]

(1) Universidad de la Frontera (UFRO), Centre of Reproductive Biotechnology (CEBIOR), Faculty of Medicine, Temuco, Chile. (2) UFRO, Doctoral Program in Applied Cellular and Molecular Biology, Faculty of Agriculture and Environmental Sciences, Temuco, Chile. (3) UFRO, Department of Agricultural Production, Faculty of Agriculture and Environmental Sciences, Temuco, Chile. (4) UFRO, Department of Agricultural Sciences and Natural Resources, Faculty of Agriculture and Environmental Sciences, Temuco, Chile

Background. The efficiency of conventional intracytoplasmic sperm injection (ICSI) in cattle is low and requires long incubation periods with activating compounds1,2, which can be detrimental to the embryos. Piezoelectric ICSI (Piezo-ICSI) without activation can optimise in vitro embryo production, as it is a faster and simpler procedure to perform3. The objective of the present study was to evaluate the pre-implantation development and quality of bovine embryos generated by both techniques. For this, fertilisation rates, embryo development and quality of bovine embryos generated by Piezo-ICSI and ICSI were compared. Methods. Embryos were generated by both injection techniques. One group was cultured for 17 hr, fixed, stained with Hoechst and observed under a fluorescence microscope to assess the progress of pronuclear formation (fertilisation). Another group of embryos was cultured until day 8 to assess the cleavage (day 3) and blastocyst formation rates (day 8). A parthenogenetic control was included for this assay. Some blastocysts were used to assess embryo quality, according to the total cell number, by fixing and staining the embryos with Hoechst. ICSI embryos and parthenotes were activated with ionomycin and anisomycin. All procedures were approved by the Scientific Ethical Committee of Universidad de la Frontera. Results were analysed according to the chi-square test ($p<0,05$). Results. No significant differences in fertilisation or embryo development rates were observed between the two injection techniques. Piezo-ICSI embryos have a higher cell number than ICSI embryos. Conclusions. Results indicate that Piezo-ICSI generates embryos with a higher number of cells than ICSI. If these results are confirmed, Piezo-ICSI could be an alternative to generate embryos of higher quality than ICSI, more simply and reliably, as it does not require exogenous activation, reducing the probability of obtaining parthenogenetically activated embryos. Genetic analysis in progress are still necessary to confirm the embryo quality data.

Keywords: ICSI, Piezo-ICSI, embryo development

Financing: Funding: FONDECYT 1201166, National PhD Scholarship ANID N°21191408

Acknowledgments: Acknowledgements: Frigorífico Temuco S.A., Temuco, Chile

Knockout of β-casein gene in MAC-T cell line using CRISPR-Cas9.

Valentina Huaiquimil[1], Erwin Muñoz[1,2], Fernanda Fuentes[1,2], Maria Elena Arias[1,3], Ricardo Felmer[1,4]

(1) Universidad de la Frontera, Centre of Reproductive Biotechnology (CEBIOR), Faculty of Medicine, Temuco, Chile. (2) Universidad de la Frontera, Doctoral Program in Applied Cellular and Molecular Biology, Faculty of Agriculture and Environmental Sciences, Av. Francisco Salazar #01145, Temuco, Chile. (3) Universidad de la frontera, Department of Agricultural Production, Faculty of Agriculture and Environmental Sciences, Av. Francisco Salazar #01145, Temuco, Chile. (4) Universidad de la Frontera, Department of Agricultural Sciences and Natural Resources, Faculty of Agriculture and Environmental Sciences, Av. Francisco Salazar #01145, Temuco, Chile

Background. The β-casein (CSN2) constitutes 28% of the total protein in cow's milk, can cause food allergy1. Therefore, the aim of this research was to design a plasmid containing the CRISPR-Cas9 system to edit the bovine CSN2 gene. In addition, selection and a reporter genes were added to this plasmid to select cells presumably edited for antibiotic resistance, and to separate them by cell sorting. Methods. A construct called pCEB was designed (see Fig.1) containing a cloning site for gRNAs and their expression, coding genes for the Cas9 enzyme, EGFP and blasticidin as resistance antibiotic. Then, using the Benchling platform, 4 gRNAs targeting different exons of the CSN2 gene were designed. The 4 gRNAs were cloned separately into the pCEB vector. Subsequently, the pCEB vector, containing each of the gRNAs, was transfected into a bovine mammary gland-derived cell line (MAC-T) to knock out the CSN2 gene. All animal experiments were conducted in accordance withthe Scientific Ethics Committee of the Universidad de La Frontera (Act Nº 057/2016). Results. The correct design of the pCEB gene construct was confirmed by PCR and transfection into MAC-T cells, as these cells expressed EGFP reporter protein and showed resistance to blasticidin. Additionally, edition of CSN2 gene could be confirmed by E1T7 assay, validating that the CRISPR-Cas9 system in the pCEB vector is functional and that the 4 gRNAs were correctly designed. Conclusions. We confirmed the knock-out of β-casein gene in MAC-T cells confirming the correct design of the pCEB plasmid. Future research will focused on the effect of this β-casein-edited gene at the translational level and will look at gene editing of pre-implantation bovine embryos to confirm the feasibility of generating modified embryos by this technique.

Keywords: CRISPR-Cas9 system, bovine ß-casein, plasmid CEB

Financing: This research was funded by the FONDECYT Project #1201166, CONICYT, Chile and the National Doctoral Scholarship ANID #21191434, Chile.

Advanced maternal age affects senescence and matrix metalloproteinase 2 in maternal-embryo interface during placenta formation

María Laura Leonardi[1], Lautaro Recchia[1], Cintia Romina Gatti[1], Alicia Jawerbaum[1], Romina Higa[1]

(1) Centro de Estudios Farmacológicos y Botánicos (CEFyBO-CONICET), Laboratorio de Reproducción y Metabolismo, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires, Argentina

Background: Pregnancy at Advanced Maternal Age (~35 years, AMA) induces obstetric complications and neonatal adverse outcomes. During early pregnancy, an optimal decidual function is essential for a correct placenta and embryo development. MMP2, a protease of senescence-associated secretory phenotype (SASP), have an important role in tissue remodeling during trophoblast invasion and embryo morphogenesis. We have previously observed that decidua of a rat AMA model showed increased MMP2 and embryonic growth impairments. Here, our aim was to evaluate in the decidua from AMA rats, the localization of 4HNE (marker of lipoperoxidation) and mRNA levels of p21 (marker of senescence) during placenta formation. Also, to evaluate mRNA levels of p21 and MMP2 in the placenta and the embryo from AMA rats together with embryonic ROS levels. Methods: 3 months old (Control) and 10 months old (AMA) Wistar rats were mated with young males (CICUAL approved by resolution Nº4081/4-2021). Decidua, placenta and embryos were obtained on day 12 of pregnancy. mRNA levels of p21 and Mmp2 were measured by RT-qPCR (n=8), 4-HNE by immunohistochemistry (n=5) and ROS by DCF-DA probe (n=7). Results: The decidua of AMA showed increased mRNA levels of p21 (+54%, $p<0.05$) and increased 4HNE immunostaining in the mesometrial region. In contrast, p21 (-27%, $p<0.01$) and Mmp2 (-26%, $p<0.05$) were reduced in the placenta of AMA rats. Embryos from AMA group showed decreased mRNA levels of Mmp2 (-33%, $p<0.05$) but no changes in p21 and embryonic ROS levels between groups. Conclusion: In AMA rats, the higher senescence in decidua increases SASP-molecules as MMP2 that, together with it increased oxidative status may affect placenta formation. Indeed, in the placenta the decreased p21 and MMP2, needed for trophoblast invasion, suggest that placentation is altered. Importantly, embryos showed reduced MMP2 probably related to the embryonic developmental impairments previously observed.

Keywords: ADVANCED MATERNAL AGE, MMP2, P21, CELLULAR SENESCENCE

Financing: Agencia Nacional de Promoción Científica y Tecnológica of Argentina (PICT 2021 GRF TI 00166) and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 2021-2023 GI 11220200100472CO).

Change in LDL receptor distribution in placentas from mothers with physiological and supraphysiological hypercholesterolemia

Andrea Morales Garrido[1], Tamara Meleñir[1], Nicolas Riquelme[1], Macarena Aguilera[1], Andrea Leiva[1]

(1) Universidad San Sebastián, Facultad de Medicina y Ciencia, Lota 2444, Santiago, Chile

Background: Maternal physiological (MPH) and supraphysiological (MSPH) hypercholesterolemia (MPH) occurs in pregnancy in response to the fetal requirements. MSPH associates with increased maternal LDL levels and arterial fatty streaks in the offspring (1). The levels of cholesterol and LDL are similar in MPH and MSPH offspring, suggesting changes in the maternal-fetal cholesterol traffic mediated by syncytiotrophoblast cells (2). LDL receptor (LDLR) endocytosis and recycling are crucial processes for cholesterol metabolism; however, the placental expression and regulation of clue components for this cellular process. Aim: to determine the expression of proteins required for a proper LDL endocytosis and recycling in MPH and MSPH placentas.

Methods: Maternal serum (n=24) and placental tissue (n=24) were classified as MPH or MSPH according to cholesterol levels (ethics approval #180810004, Pontificia Universidad Católica de Chile). The abundance and localization of proteins associated to LDLR endocytosis and recycling (α and β adaptin, transgelin, Blos-1, CCD93, clathrin, PCSK9, SNX17, and LDLR), were determined by Western Blot and immunofluorescence. The expression of the LDLR gene was evaluated by qPCR. Values are mean ± standard error mean. Data were analyzed by t-test and considered significant when $p<0.05$.

Results: No changes in protein abundance or expression of LDLR were determined in MPH and MSPH placentas. However, we find differences in the location of LDLR in syncytiotrophoblast, showing it to be predominantly apical in MSPH compared to MPH. Additionally, we described for the first time that all the studied proteins are present in the placental tissues and that α adaptin is reduced in MSPH placentas meanwhile clathrin present changes in its cellular localization.

Conclusions: Proteins required for a proper LDLR endocytosis and recycling are expressed in the placental tissue. LDLR, α adaptin and clathrin are differentially expressed in MSPH placentas, which can lead to changes in maternal-fetal LDL traffic and cholesterol metabolism.

Keywords: LDL receptor, Syncytiotrophoblast, Hypercholesterolemia

Financing: FONDECYT Regular 1230527

Effect of endometriosomes on modulation of development and quality of bovine embryos produced by IVF.

Erwin Muñoz[1,2], Valentina Huaiquimil[1], Felipe Pérez[1,2], Fernanda Fuentes[1,2], Ricardo Felmer[1,3], María Elena Arias[1,4]

(1) Universidad de la Frontera (UFRO), Centre of Reproductive Biotechnology (CEBIOR), Faculty of Medicine, Temuco, Chile. (2) PhD Program in Applied Cell. and Molec. Biology, (3) Dept. of Agricultural Sciences and Natural Resources and (4) Dept. of Agricultural Production, Faculty of Agriculture and Environmental Sciences, UFRO, Temuco, Chile.

Background. In vitro fertilization (IVF) efficiency in cattle is still low. Small extracellular vesicles (sEVs) mediate communication between maternal cells and the embryo, leading to changes in the transcriptome, proteome, and epigenome of the embryo1. The sEVs secreted by endometrial cells (endometriosomes) have been shown to benefit the development and quality of embryos produced in vitro in different species1. In this study, we evaluated the effect of sEVs secreted in vitro by bovine endometrial stromal (BESC) and epithelial (BEEC) cells on the development and quality of bovine embryos produced by IVF. Methods. BESC and BEEC were cultured for two days. Then, endometriosomes were isolated and purified from the media conditioned by both cell types. Isolation was performed by polymer-based precipitation, and purification was performed by ultrafiltration with a 10 nm nanopore polycarbonate filter. IVF2 produced embryos. One group was cultured in KSOM containing endometriosomes from BESC (BESC-E), and the other in KSOM containing endometriosomes from BEEC (BEEC-E). A control of embryos cultured in KSOM free of endometriosomes was included. Cleavage (day 3) and blastocyst development (day 7) rates were assessed. The results were analyzed using the chi-square and Fisher's exact tests ($p<0.05$). All animal experiments were conducted in accordance with the Scientific Ethics Committee of the Universidad de La Frontera (Act Nº 057/2016). Results. Endometriosomes of both cell types were successfully isolated. Table 1 shows that BEEC-E significantly increased embryonic development compared to the control. In addition, immunofluorescence of the embryos shows an apparent higher number of cells in the inner cell mass in BESC-E and BEEC-E compared to the control. However, further replicates are needed to validate the latter results. Conclusions. Supplementing the embryo culture medium with BEEC-E may improve the development and quality of bovine embryos generated by IVF. However, further studies are needed to elucidate the mechanisms underlying this improvement.

Keywords: endometrial cell culture, bovine embryos, IVF, endometriosomes (sEVs)

Financing: Funding. This research was funded by the DI-UFRO Project DI20-0062 and the National Doctoral Scholarship ANID #21191434, Chile. Acknowledgements: Frigorífico Temuco for providing us with samples of bovine tissue.

Repetitive maternal restraint stress during pregnancy impairs cortical fetal neurogenesis.

Sebastian Oyarce Pezoa[1,2], Mario Sánchez Rubio[1], Daniela Corvalán Bustos[1], Maxs Mendez Ruette[1,2,3], Luis Federico Bátiz[1,3,4]

(1) Neuroscience Program, Center for Biomedical Research and Innovation (CIIB), Universidad de los Andes, Santiago, Chile. (2) Ph.D. Program in Biomedicine, Faculty of Medicine, Universidad de los Andes, Santiago, Chile. (3) IMPACT Center of Interventional Medicne for Precision and Advanced Cellular Therapy, Santiago, Chile. (4) School of Medicine, Faculty of Medicine, Universidad de los Andes, Santiago, Chile

Background: Psychological distress during pregnancy or prenatal stress (PS) increases the risk of poor neurodevelopmental outcomes in the offspring, resulting in long-lasting consequences. However, PS-induced alterations in fetal neurogenic processes (i.e., proliferation, differentiation, and maturation) are poorly understood. In this work, we focused on understanding morphological and molecular changes induced by repetitive maternal restraint stress in cortical fetal neurogenesis, using a rat model. Methods: Pregnant rats were split at embryonic day (E)0.5 in control and stress (repetitive restraint) groups. Between E8.5 and E17.5, rats from the restraint group were confined daily to a wired box of 8x8x23cm for 2h. Both groups were euthanized at E15.5 (n=5/group) or E18.5 (n=5/group), followed by extraction and recovery of embryos. Proliferation/differentiation assays were performed by intraperitoneal injection of BrdU (50mg/kg) at E14.5 and evaluation at E15.5 and E18.5. In addition, the process of neuronal differentiation and maturation in the fetal brain cortex was evaluated by qRT-PCR, WB assays, and immunofluorescence, using cell specific markers. The morphological characterization of developing brains was performed by immunofluorescence. All procedures were approved by Universidad de los Andes Bioethical committee and followed NIH Guide for Care and Use of lab Animals. Results: PS induce changes in the fetal cortical neurogenic process at different levels. We observed a decrease in the number of intermediate progenitor cells at E15.5 and E18.5. On the other hand, differentiation assays suggest a premature neuronal differentiation. Finally, the expression of maturation markers such as MAP2 showed a significant decrease at E18.5, suggesting a PS-induced impairment in neuronal maturation processes. Conclusions: PS induces mayor alterations in fetal brains characterized by a decrease in neural progenitors at early neurogenic stages, acceleration of neuronal differentiation, and a decrease of neuronal maturation markers at late stages of fetal neurodevelopment.

Keywords: Prenatal Stress, Neurogenesis, Fetal Brain Development

Financing: FONDECYT 1211384

Small Extracellular Vesicles as a Promising Candidate for Enhancing in vitro maturation of Bovine Embryos

Felipe Pérez-García[1,2], Erwin Muñoz Acuña[1,2], Ricardo Felmer Dörner[2,3], Maria Elena Arias Cea[2,4]

(1) Doctorate in Science with Specialization in Applied Molecular and Cell Biology, Universidad de La Frontera, Temuco, Chile. (2) Laboratory of Reproduction, Centre of Reproductive Biotechnology (CEBIOR-BIOREN), Universidad de La Frontera, Temuco, Chile. (3) Department of Agricultural Sciences and Natural Resources, Faculty of Agriculture and Environmental Sciences, Universidad de la Frontera, Temuco, Chile. (4) Department of Animal Production, Faculty of Agriculture and Environmental Sciences, Universidad de La Frontera, Temuco, Chile.

Background. Small extracellular vesicles (sEVs) are involved in cell-cell communication through the transport of various biomolecules1. In the area of animal reproduction, recent studies have demonstrated the implication of sEVs in the development of gametes and embryos in different mammalian species; however, very few have proposed the use of sEVs as a strategy to improve assisted reproduction techniques, such as in vitro embryo production2. The study aimed to isolate and characterize sEVs from follicular fluid for their utilization in the in vitro maturation (IVM) of bovine oocytes. Additionally, it investigated the effect of sEVs supplementation on gene expression related to oocyte quality post-IVM. Methods. Isolation of sEVs from follicular fluid was performed by differential centrifugation followed by ultrafiltration and ultracentrifugation. The sEVs obtained were characterized by particle size analysis (DLS), electron microscopy (SEM-STEM) and western blot analysis for the sEVs markers ALIX and CD63. The expression levels of BAX, BCL2, HSF1, and BMP15 genes were quantified using RT-qPCR. Results. The sEVs fraction obtained exhibited a particle size of 119.9 ± 30.29 nm. ALIX and CD63 markers were detected in the sEVs samples, whereas these markers were not detected in the follicular cell samples. The expression analysis is currently underway, and the results will be available for presentation. Conclusions. This preliminary study successfully isolated and characterized sEVs from follicular fluid for potential use in bovine oocyte in vitro maturation. The sEVs fraction obtained exhibited appropriate particle size, and the presence of sEVs markers confirmed their isolation. While the expression analysis is still ongoing, these results contribute to our understanding of sEVs involvement in reproductive processes and highlight their potential for improving assisted reproduction techniques. Further investigation is needed to fully explore the benefits of sEVs supplementation on oocyte quality and in vitro embryo production.

Keywords: extracellular vesicles, bovine oocytes, in vitro maturation

Financing: ANID National Doctoral Scholarship N° 21191434.

Neural tube defects are sex-dimorphic and dependent on the genetic background in SR-B1 KO embryos

Camila Romero-Muñoz[1], Gabriela Belledonne[1,2], Fujiko Saavedra[1,3], Patricia Romo-Toledo[1], Dolores Busso[1,4]

(1) Universidad de los Andes, Laboratory of Reproductive Biology, Center for Biomedical Research and Innovation, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, Ph.D Program in medical sciences, Faculty of Medicine, Santiago, Chile. (3) Universidad de los Andes, Ph.D Program in Biomedicine, Faculty of Medicine, Santiago, Chile. (4) Universidad de los Andes, IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background: SR-B1 is an HDL receptor expressed in the yolk sac and placenta. SR-B1 KO embryos exhibit a high prevalence of cranial neural tube defects (NTD) with a sex bias toward females. We currently maintain two colonies of SR-B1 KO mice. Colony SR-B1 was always maintained in a 129:B6 background. Jackson Laboratory recently donated a second colony to us, which was backcrossed to B6 (SR-B1/J). Methods: Embryos were dissected at embryonic day 9.5 (E9.5) and neural tube closure and developmental progress were registered. Screening and sexing of embryos were achieved by PCR using extraembryonic tissues. Results are expressed as mean±SD or proportions. $P<0.05$ were considered significant. Protocols were conducted in agreement with the National Research Council (NRC) publication Guide for Care and Use of Laboratory Animals (8th edition, 2011) and approved by the Ethics Committee from Universidad de los Andes (ID N° CEC2022030). Results: The NTD incidence SR-B1/J KO colony was higher than SR-B1colony [92.3% (12/13) vs. 22.9% (22/96)], respectively; p=0.0001, Fisher's exact test]. The incidence of NTDs in the SR-B1/J colony was higher in females [54.6% (6/11) vs. 7% (1/14) in males; p=0.0213], as previously observed in the SR-B1 colony. The proportion of developmental delayed KO embryos was higher in the SR-B1/J colony, but this difference did not reach significance [30.77% (4/13) vs. 10.42% (10/96)] in SR-B1 colony; p=0.0623, Fisher's exact test]. However, the mean somite n° was lower in the SR-B1/J-KO embryos (13.8±5.7 vs. 18.6±4.1 vs. SR-B1; p=0.0031, Mann-Whitney test). Conclusions: SR-B1 KO embryos from the two colonies show significant phenotypic differences: there is an increase NTD incidence and smaller embryos in SR-B1/J. Both colonies show sex-dimorphic NTD. These results show that the genetic background may modify the risk of NTD, allowing for future studies to identify genetic modifiers that may contribute to understanding the etiology of NTD.

Keywords: NTD, SR-B1, Sexual Dimorphism

Financing: FONDECYT #1221376 and ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024 (to DB).

Differential distribution of lipid receptors and transporters in embryonic and extraembryonic mouse tissues during neurulation

Patricia Romo-Toledo[1], José Chávez[2], Nicolás González[2], Reyna Peñailillo[1], Dolores Busso[1,3], Gabriela Belledonne[1]

(1) Universidad de los Andes, Laboratory of Reproductive Biology, Centre for Biomedical Research and Innovation (CIIB), Santiago, Chile. (2) Universidad San Sebastián, Medical Technology career, Faculty of Medicine, Santiago, Chile. (3) Universidad de los Andes, IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

In mouse embryonic development, organogenesis begins with neurulation at gestational day 9.5 (E9.5). The provision of cholesterol and lipophilic vitamins are relevant for the correct neural tube closure since they promote morphogenesis, prevent damage caused by oxidative stress and maintain the membranes' integrity. Indeed, deficiency of different lipid transporters and lipoprotein receptors leads to neural tube defects. Early embryonic nutrition occurs through the absorption of secretions from the uterine glands through early extraembryonic tissues, i.e., the parietal and visceral yolk sac (YS). This work aimed to describe the expression and localization of different lipid transporters and receptors in the embryo and YS. Sections of C57BL/6 conceptuses at E9.5 and dissected embryonic and extraembryonic tissues were analyzed through immunohistochemistry (IHC) and western blot, respectively. Publicly available RNAseq databases from E9.5 mouse visceral YS (Cindrova-Davies 2017), parietal YS (Hannibal 2014) and embryos (Santander 2018) were used to determine expression levels in each tissue. Protocols were conducted in agreement with the National Research Council (NRC) publication Guide for Care and Use of Laboratory Animals (8th edition, 2011) and approved by the Ethics Committee from Universidad de los Andes (ID N° CEC2022030). Our results show that the expression of lipoprotein receptors is low in embryos (less than 20 rpkm and null staining in IHC). By contrast, extraembryonic tissues show significant expression of some receptors. In the vYS, a high expression of two multiligand receptors that form a complex, cubilin (354,6 rpkm) and amnionless (125 rpkm), was consistent with WB data and localization by IHC. A very high expression of the HDL receptor SR-B1 (20.330,8 rpkm) and very high signals in both WB and IHC were detected in trophoblasts from the pYS. The differential expression and location of lipoprotein receptors in extraembryonic tissues during early development suggest that these tissues have coordinated functions to support organogenesis.

Keywords: Embryonic development, metabolism, neurulation

Financing: FONDECYT 1221376 and ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT (to D.B)

Impact of Combined Activation Treatments on Bovine Embryonic Development and Cell Regulators MPF and MAPK

Cecilia Valencia Robles[1], Felipe Pérez[1], Carola Matus[4], Ricardo Felmer[1,2], Luis Aguila[1], María Elena Arias[1,3]

(1) Laboratory of Reproduction, Centre of Reproductive Biotechnology (CEBIOR-BIOREN), Faculty of Medicine, Universidad de La Frontera (UFRO), Temuco, Chile. (2) Dept. of Agricultural Sciences and Natural Resources and (3) Dept. of Animal Production, Faculty of Agriculture and Forestry Sciences, UFRO, Temuco, Chile. (4) UFRO, Dept. of Basic Sciences, Faculty of Medicine, Temuco, Chile.

Previously, we demonstrated that the use of anisomycin (ANY) improves the rates and quality of embryos obtained through ICSI, SCNT, and parthenogenesis compared to commonly used chemical compounds such as cycloheximide (CHX) and 6-dimethylaminopurine (DMAP).1,2. The aim of this study was to evaluate and compare oocyte activation treatment by combinations of protein synthesis inhibitors (ANY and CHX) and protein phosphorylation inhibitor DMAP (ANY; ANY+CHX; ANY+DMAP; ANY+CHX+DMAP; and CHX+DMAP), it was evaluated early embryonic development, expression and phosphorylation of the components of the Maturation Promoting Factor (MPF) and MAPK pathway by western-blot assay. The results showed that no differences were observed between treatments in terms of cleavage rates (91.6% to 98.6%) and blastocyst formation (37.4% to 50.9%). None of the treatments tested altered the expression of CCB1, CDK1, or phosphorylation of CDK1 residues. However, treatments ANY and ANY+CHX showed a decreased expression of ERK1/2 protein compared to ANY+DMAP; ANY+CHX+DMAP; and CHX+DMAP ($p<0.05$). Additionally, the treatments ANY+DMAP; ANY+CHX+DMAP; and CHX+DMAP showed a decrease in p-ERK1/2 compared to treatments ANY and ANY+CHX ($p<0.05$). Therefore, the inactivation of MPF with all the treatments used in this study to not occur through a decrease in CCB1, and considering that p-CDK1 in Thr161 and Thr14-Tyr15 residues remain balanced, it can be presumed that MPF is in its inactive form (preMPF). Furthermore, protein synthesis inhibitors, lead to a decrease in the level of ERK1/2. On the other hand, DMAP combined with ANY, CHX, or both, decreases MAPK phosphorylation compared to treatments with protein synthesis inhibitors. In conclusion, our results indicate that the aforementioned treatments promote the activation of bovine oocytes in an ERK1/2-dependent manner. Considering the favorable results in cleavage rates and blastocyst formation, it would be appropriate to analyze the embryonic quality of the parthenotes obtained with these treatments.

Keywords: Parthenogenic activation, inhibition of protein synthesis and phosphorylation

Financing: FONDECYT-1181453, Doctorado Nacional-21181741 ANID-Chile. Acknowledgments: Frigorífico Temuco for the supply of ovaries.

Altered expression of specific miRNAs in domestic cat blastocysts cultured without the zona pellucida

Daniel Veraguas-Dávila[1,2], Camila Zapata-Rojas[3], Diego Caamaño[1], Darling Saéz-Ruiz[1], Yazmín Vásquez[1], Fernando Saravia[1], Fidel Ovidio Castro[1], Lleretny Rodríguez-Alvarez[1]

(1) Universidad de Concepción, Departamento de Ciencia Animal, Facultad de Ciencias Veterinarias, Chillán, Chile. (2) Universidad Católica del Maule, Departamento de Ciencias Agrarias, Escuela de Medicina Veterinaria, Facultad de Ciencias Agrarias y Forestales, Curicó, Chile. (3) Universidad Andrés Bello, Facultad de Ciencias de la Vida, Escuela de Medicina Veterinaria, Viña del Mar, Chile

Background. The domestic cat is a valuable model for the development of assisted reproductive technologies in endangered felids. However, domestic cat embryos cultured without the zona pellucida are not able to implant (1). The reason for this lack of implantation is still unknown. The objective of this study was to evaluate the expression of specific miRNAs in domestic cat embryos cultured without the zona pellucida. Methods. Procedures were approved by the ethics committee of the Universidad de Concepción (CEBB 616-2019). Experimental groups: 1) domestic cat embryos generated by IVF (ZI group). 2) domestic cat embryos generated by IVF and cultured without the zona pellucida (ZF group). In the ZF group, after IVF the zona pellucida was removed using pronase and embryos were cultured in microwells. Cleavage, morula, and blastocyst rates were estimated at days 2, 5 and 7 of IVC. Blastocysts were subjected to RT-qPCR analysis using the standard curve method to evaluate miRNAs related to embryo development: miR-21, miR-24, mi25, miR-29, miR-96, miR-98, miR-196, miR-199, miR-130. Meanwhile, miR-103 and miR-191 were used as internal control (2). The Wilcoxon non-parametric test was used for analysis ($P < 0.05$). Results. No differences (% Mean ± SD) were observed in the cleavage: ZI = 201/455 (44.2 ± 18.1), ZF = 240/585 (41.0 ±19.9); morula: ZI = 95/201 (47.3 ± 14.1), ZF = 122/240 (50.8 ± 26.5); and blastocyst rates: ZI = 56/201 (27.9 ± 9.2), ZF = 86/240 (35.9 ± 19.7). However, the relative expression of miR-21, miR-25, miR-29 and miR-199 was higher in ZF blastocysts than in their ZI counterparts. Furthermore, the relative expression of miR-96 was lower in ZF blastocysts. Conclusion. Culture of domestic cat embryos without the zona pellucida did not affect in vitro development, but modified the expression of miR-21, miR-25, miR-29, miR-199 and miR-96 in blastocysts.

Keywords: felid embryos, in vitro fertilization, gene expression analysis.

Financing: Funding. ANID FONDECYT POSTDOCTORADO 3200352. Acknowledgments: Acknowledgments. Laboratorio de Biotecnología Animal, Departamento de Ciencia Animal, Universidad de Concepción, Chillán.References.Veraguas-Davila et al. (2020). Theriogenoloy. 174(1) 36-46.Mahdipour et al. (2015). BMC Dev Biol. 15 (1) 25.

Exposure to particulate matter from wood combustion smoke produces vascular changes in the placental disc

Francisca Villarroel[1,2], Paulo Salinas[1], Nikol Ponce[3,4,5], Fernando Gomez[6], Cristian Muñoz[6], Eder Ramirez[7], Francisco Nualart[7,8]

(1) Lab. Morfología Animal y Experimental, I. de Biología, F. de Ciencias, Pontificia Universidad Católica de Valparaíso (PUCV), Valparaíso, Chile. (2) MSc. Program in Biological Sciences, PUCV, Valparaíso, Chile. (3) PhD Program in Morphological Sciences, Universidad de La Frontera, Chile. (4) Centro de Excelencia en Estudios Morfológicos y Quirúrgicos, Universidad de La Frontera, Chile. (5) ANID-Doctorado #21190655. (6) Lab. of Genetics and Molecular Immunology, I. of Biology, F. Ciencias, PUCV, Valparaíso, Chile. (7) Lab. of Neurobiology and Stem Cells, F. Biological Sciences and (8) Center for Advanced Microscopy CMA BIO-BIO, University of Concepcion, Concepcion, Chile.

Background: Fine particulate matter (PM2.5) represents the main air pollutant affecting the population and is considered a threat to the health of pregnant females and the fetus. The placenta requires normal vascular growth and remodeling to transfer oxygen and nutrients. Angiogenesis is crucial during embryonic development for tissue vascularization, with hypoxia being the main triggering factor of this process. This study proposes that variations in placental vascular morphophysiology during gestation, induced by hypoxia, contribute to the reduction in fetal size associated with maternal exposure to PM2.5 from wood smoke. We evaluated the effects of PM2.5 exposure on vascular morphology and the expression of angiogenic factors (HIF1A-PIGF-VEGF-Flt1-Kdr) in the placental disc (DP). Methods: Experiments with rats were carried out in accordance with the Scientific Ethics Committee of UFRO (Act-122/20). Exposure chambers received filtered air (AFgroup;n=24;control) and unfiltered air (ANFgroup;n=24). Female Sprague-Dawley rats were exposed to PM2.5 during the pregestational and/or gestational stages. At post-fertilization (21 days), DP was obtained through cesarean section. In DP, oxygen diffusion capacity was calculated, and the expression of angiogenic factors was evaluated using qPCR and immunohistochemistry. Results: In the ANF group exposed to PM2.5 during the pregestational and/or gestational stages, a decrease in fetal weight ($p=0.0022$) and crown-rump length ($p=0.0005$), theoretical l($p<0.0001$) and specific ($p<0.0001$) diffusion capacity of oxygen, and expression of HIF1A ($p<0.0001$) were observed. In the ANF group exposed to PM2.5 exclusively during the pregestational stage, the placental gene expression of Flt1 ($p<0.0001$), Kdr ($p<0.0001$), and PIGF($p=0.0314$) increased. Additionally, in the labyrinth region (DP), the expression of all angiogenic factors increased ($p<0.000$; Figure 1). Conclusions: Exposure to PM2.5, whose main source is wood smoke, generates prolonged hypoxic conditions, altering DP angiogenesis, and reducing oxygen diffusion capacity between the mother and fetus, thereby impacting fetal size. Financing: Fondecyt N°1120077

Female Reproduction

Regulation of endometrial senescence by mitochondrial function/dysfunction: potential role in idiopathic recurrent pregnancy loss

Macarena Aguilera Olguín[1], María José Yañez Henríquez[1], Jaime Gutierrez[1]

(1) Universidad San Sebastián, Faculty of Medicine and Science, Santiago, Chile

Background. Recurrent pregnancy loss (RPL) is defined as the spontaneous loss of 2 or more pregnancies, affecting »2% of pregnant women. Up to 50% of patients with RPL have no clearly defined etiology, which is known as idiopathic RPL. Decidualization of the endometrial stromal cell by a process of mesenchymal to epithelial transition is a key step for pregnancy success. Perturbations in this process, termed decidualization, is associated whit miscarriage, however the underlying mechanisms are poorly understood. Two sub-populations of decidual cells (DC) can be found, the ones whit anti-inflammatory and proliferative phenotype, known as DC, which are present along pregnancy and DC-senescent cells, with pro-inflammatory and non-proliferative phenotype which are transiently present and required during implantation. However, increased number and persistent accumulation of DC-senescent cell is observed in RPL, which could be associated to this reproductive problem. Although, cell senescence is classically induced by stress factors, such as mitochondrial dysfunction (MD). There isn't evidence that link decidual MD with the development of RPL pathophysiology. Therefore, we propose that "MD promotes endometrial senescence in a context of idiopathic RPL".Methods. Endometrial stromal cells were isolated from menstrual fluid and treated with electron transport chain inhibitors to induce MD. The localization of ATP, ROS, mitochondrial proteins and mitochondria was determined, and the induction of senescence phenotype markers will be determined.Results.We project to find an increase in the senescence phenotype in cells with induced MD, which we also propose to evaluate in samples with idiopathic RPL.Conclusions. Actually, the cellular mechanisms in RPL are unknown, we expect to find a link between DM and senescence. In case of identifying this link, it's proposed as a tool for a possible diagnosis of RPL that doesnt' currently exist.Keywords: Idiopathic recurrent pregnancy loss, senescence phenotype, mitochondrial function, mitochondrial dysfunction.

Financing: Fondecyt Regular 1221362 & 11200592, ANID/Doctorado nacional 21221232

Maternal Mgat1a: A regulator of cortical granule biology during the oocyte-to-egg transition

Constanza Aguirre-Campos[1,3], Felipe Aguilera[2,3], Mary C Mullins[4], Ricardo Fuentes[1,3]

(1) Department of Cell Biology and (2) Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile. (3) Developmental Processes Group, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile. (4) Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, USA.

Background: Infertility is influenced by several factors. One of them is poor egg quality during activation, which affects females of all ages. However, there is a lack of biomarkers for assessing egg quality. The most commonly used parameter to determine egg quality is the modification of the egg's surface, which prevents the entry of multiple sperm. Cortical granules (CGs) are secretory vesicles that release their contents upon egg activation, leading to the expansion of the perivitelline space. While CG biology has been extensively studied in invertebrate organisms, the genetic program responsible for preventing polyspermy in vertebrates remains unknown. Phenotyping approaches have shown that zebrafish eggs produced by mgat1a mutant mothers show smaller CGs and perivitelline space. Methods: We identified the zebrafish mgat1asa9475 mutant through a phenome-wide screen. We employed microscopy techniques, CG staining, histology, and in silico analysis to investigate the morphological and molecular features of oocytes, eggs, and early embryos. Results: Eggs produced by mutant mothers displayed altered CG size and dynamics, and failed to expand the perivitelline space. Due to limited space during development, the embryos showed abnormal axis determination. Analysis of the predicted structure and function of maternal Mgat1a suggests its involvement in the N-glycosylation pathway, and the mutation leads to the loss of 92 C-terminal amino acids. Conclusion: The presence of small and numerous CGs after fertilization indicates compromised biosynthesis and exocytosis. This impacts the expansion of the perivitelline space. Maternal Mgat1a regulates N-glycosylation prior to fertilization, with its function residing in the C-terminal region. The mgat1a mutant serves as a valuable tool for understanding the N-glycome and its role in shaping the female gamete and its activation. Furthermore, it represents a significant advancement in our understanding of polyspermy prevention and the achievement of successful in vitro fertilization.

Keywords: Polyspermy, Egg activation, Mgat1a

Financing: FONDECYT de Iniciación 11201118 and VRID 20.031.117-M

"*The cellular distribution of RECK is regulated by its glycosylation's in human first trimester trophoblasts.*"

Nicolas Daniel Alarcon Merino[1], Jaime Gutierrez[1], Rodrigo Escalona[1], Lukas Salazar[1]

(1) CSDL, Medicina y salud, Medicina, Providencia, Región Metropolitana, Santiago, Chile

Background: Preeclampsia (PE), a severe pregnancy specific syndrome, is believed to originate from incomplete remodeling of uterine arteries due to insufficient invasion by extravillous trophoblasts. We found that the expression of RECK, a plasma membrane glycoprotein (GPI-anchored), which plays critical role as matrix metalloproteinases (MMP) and cell invasion inhibitor, is augmented in trophoblast cells from the severe form of PE. We found that RECK inhibit trophoblast invasion by a MMP dependent mechanism. By high-resolution SDS-PAGE and western blot analysis we found three bands of different molecular weights corresponding to RECK. One possible explanation is a differential glycosylation of the same protein. Which could also affect is cellular distribution and function. Objective: To determine the glycosylation of RECK and its potential role in defining its membrane localization in first trimester trophoblasts. Methods: RECK glycosylation and plasma membrane localization was analyzed in human first trimester cell line, HTR8/Svneo, by treating cell lysates with PNGase (removal of N-linked oligosaccharides from glycoproteins) or the treatment of cultured cells with tunicamycin (an inhibitor of N-glycosylation). Cell cultures were also treated with Phosphatidylinositol-specific phospholipase C (PIPLC), which release GPI-anchored proteins from the plasma membrane. Western blot of cell lysates and immunofluorescence analysis were performed. Results: PNGase treatment abolish the presence of higher, ~130kDa band, increasing at the same time, the intermediate, ~120kDa band. No changes in the lower, ~100 kDa band. Tunicamycin treatment, reduced the 130 kDa band without effects on the other (120 and 100 kDa bands). PIPLC treatment revealed that only the 130 and 100 kDa bands were forms of RECK present on the cell surface. Conclusions: RECK exhibits 3 bands of different molecular weights in HTR8/SVneo cells.

Keywords: Preeclampsia, RECK, glycosylation

Financing: FONDECYT regular 1221362.

Increased NPC1 and STARD3 expression in placentas from women with maternal supraphysiological hypercholesterolemia (MSPH) promotes lysosomal and mitochondrial dysfunction

Gabriela Arenas[1], Silvana Zanlungo[2], Andrea Leiva[1], María José Yañez[1]

(1) Universidad San Sebastian, Escuela de Tecnología Médica, Facultad de Medicina y Ciencia, Santiago, Chile. (2) Pontificia Universidad Catolica de Chile, Facultad de Medicina, Santiago, Chile

Background: During the progress of human pregnancy, the concentrations of total cholesterol (TC) rise to physiological (MPH) or supraphysiological (MSPH) levels (1, 2). Maternal-to-fetal cholesterol trafficking is poorly understood. Classically, after receptor-mediated uptake of LDL, the particle is transported to the endosome/lysosome pathway via NPC1 and STARD3, which transfers cholesterol from the lumen of the lysosome to the mitochondria (3). Methods: We used: i) HTR8/SVneo and BeWo cells treated with ox-LDL (50-100 mg/mL, 24 h), and ii) placentas from women with MPH or MSPH pregnancies. We evaluated the structure and function of lysosomes and mitochondria in cells exposed to increased TC levels and MSPH placentas (ethics approval #CEC2022023, Universidad de los Andes).Results: We show that treatment with ox-LDL did not change the expression of the proteins involved in cholesterol transport and in mitochondrial dynamics in HTR8/SVneo cells, however, lysosomal dysfunction occurred, reactive oxygen species (ROS) levels were lower and ATP levels did not change. In contrast, in BeWo cells treated with ox-LDL and in the placentas from women with MSPH, we found an increase in proteins involved in cholesterol transport and mitochondrial dynamics, lysosomal dysfunction, and decreased ROS and ATP content.Conclusion: MSPH is associated with changes in the structure and function of lysosomal and mitochondria.

Keywords: Pregnancy, cholesterol, lysosomal and mitochondrial dysfunction

Financing: FONDECYT N°11200592.

Cholesterol Excess Associated to Lipid Storage Abnormalities and Impaired Autophagy in Infertile SR-B1 KO Mice.

Andreina Arias[1,2,3], Fujiko Saavedra[1], Eugenia Morselli[4], Dolores Busso[1,3]

(1) Universidad de Los Andes, Center for Biomedical Research and Innovation, Program of Reproductive Biology, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, Physiological Sciences, Faculty of Biological Sciences, Santiago, Chile. (3) 3IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile (4) Universidad San Sebastian, Department of Basic Sciences, Faculty of Medicine and Sciences, Santiago, Chile

Background. Female SR-B1 KO mice are infertile. Their eggs show high spontaneous activation and death rates after ovulation1,2. WT mouse eggs loaded with methyl-β-cyclodextrin-cholesterol (MβCD-chol-eggs) undergo parthenogenic cleavage and reduced viability after activation, phenocopying SR-B1 KO eggs2. Cholesterol accumulation affects cellular lipid content, viability, and function. This work aimed to characterize global lipid content in SR-B1 KO eggs and study the status of different cell death pathways, i.e., necrosis and autophagy, in eggs with cholesterol excess. Methods. Eggs were retrieved from superovulated SR-B1 KO and WT females. Lipids were characterized by shotgun lipidomics (3 pools of 100 oocytes/genotype) (Lipotype, Germany). Autophagy was evaluated using immunofluorescence (IIF) for p62 and LC3. WT eggs were exposed to MβCD-col (0.125μM) and incubated with SrCl2 (MβCD-chol-eggs+SrCl2) to induce activation-mediated death. To assess autophagy, 250nM rapamycin (rapa) or 100nM Bafilomycin A1 (bafA1), inductor and inhibitor of autophagy, were included in the culture medium. A lactate dehydrogenase (LDH) assay was used to evaluate cytotoxicity and necrosis. Results. KO eggs were enriched in cholesterol ester (CE) (8.6±2.8%mol/sample vs. non-detectable level in WT; p=0.039) and had 0.6X lower TAG levels (p=0.045). SR-B1 KO eggs showed higher fluorescence for p62 (1.7±0.1 vs. 1.0±0.1 WT) and more Lc3B dots (569±21 vs 374±38 WT, n=10), suggesting impaired autophagy. A tendency to lower demise was observed in MβCD-chol-eggs+SrCl2 incubated with rapa (29±8%) or bafA1 (29±13%), vs. 53±13% in MβCD-chol+SrCl2 eggs without inhibitors (n=3 experiments, n=20/each). Finally, high cytotoxicity levels were observed in MβCD-chol-eggs+SrCl2 60min after Sr addition (90±9% vs. 4±3% in non-activated MβCD-chol-eggs n=2 experiments, n=60/each). Conclusions. Our results show that cholesterol excess is associated with disruption in the proportion of storage lipids and with impaired autophagy in SR-B1 KO eggs. They also suggest that dysregulated autophagy and necrosis may contribute to MβCD-chol-eggs demise after activation.

Keywords: cholesterol excess; eggs dysfunction; infertility

Financing: ANID-FONDECYT 1221376 and ANID-Basal Impact #FB210024 grants (to DB), VRI-UC, ANID 21211964 and IMPACT PhD fellowships (to AA).

Porphyromonas gingivalis-lipopolysaccharide and high-glucose induce placental inflammation in healthy and gestational diabetes mellitus pregnant women

M. J. Bendek[1], M. L. Mizgier[1], C. Rojas[1], D. Busso[2,3], L. J. Monteiro[2,3], A. Chaparro[1]

(1) Universidad de los Andes. Laboratory of Periodontal Investigation, Centre for innovation in Biomedical Research (CiiB), Faculty of Dentistry, Santiago, Chile. (2) Laboratory of Biology of Reproduction, CiiB, Faculty of Medicine, Santiago, Chile. (3) Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Chile

Background: Periodontal bacteria, Porphyromonas gingivalis, and its lipopolysaccharide (P.g-LPS) translocate from the periodontal pocket to the placenta (1). Current evidence suggests the placenta as a source of proinflammatory mediators that further worsen glycemic control in cases of gestational diabetes mellitus (GDM) (2). We explored the effect of P.g-LPS and high-glucose, on placental inflammatory pathways and placental-extracellular vesicles (pEVs) microRNA cargo in an ex-vivo context. Methods: Term-chorionic villi explants from healthy (n=40) and GDM (n=20) pregnant women were stimulated with P.g-LPS (1 µg/mL) and high-glucose (HG,25mM). NF-κB, NLRP-3 inflammasome, and cytokines IL-6, IL-1β and TNF-α were assessed by western blot (WB), immunofluorescence, and real time-qPCR (RT-qPCR). pEVs were isolated from culture supernatant by ultracentrifugation and characterized by WB, nanoparticle tracking analysis, and transmission electronic microscopy. pEVs-miRNA cargo was analyzed by RT-qPCR. Statistical multivariate comparison analysis ANOVA was performed (alpha=0.05). Results: Ethics committee approved this project. The P.g-LPS+HG stimuli increased NF-κB phosphorylation, NLRP-3 protein expression, and NF-κB nuclear immunolocalization in placental explants from healthy pregnant women ($p=0.017$, $p<0.0001$, and $p=0.0003$, respectively), with no effect in placental explants from GDM pregnant women. P.g-LPS+HG increased IL-6, IL-1β and TNF-α mRNA expression in placental explants from healthy pregnant women ($p=0.0363$, $p=0.0132$, $p=0.0025$, respectively) and IL-1β and TNF-α in GDM explants ($p=0.0178$, $p=0.0171$, respectively). pEVs were positive to EVs-markers CD63, CD9, CD81, Syntenin-1 and ALIX and had cup-shaped morphology. pEVs concentration had non-significant differences upon stimulation. Nevertheless, P.g-LPS+HG downregulated the anti-inflammatory miRNA cargo of miR-16-5p and mir-26a-5p in placental explants from GDM pregnant women ($p=0.0341$, $p=0.0451$, respectively), which was not observed in pEVs from healthy pregnant women. Conclusions: The ex-vivo placental model from healthy and GDM pregnant women responds to P.g-LPS and HG combined stimuli by increasing the activation of inflammatory pathways (NF-κB, and inflammasome NLRP-3), and particularly in GDM cases reducing the anti-inflammatory pEVs-miRNA cargo.

Financing: ANID CHILE FONDECYT REGULAR ID1211471, ANID CHILE PhD GRANT 2019-21190319

Maternal supraphysiological hypercholesterolemia during pregnancy modulates the biology and function of placental-derived small extracellular vesicles

Claudette Cantin[1], Elisabet Manríquez[1], Andrea Leiva[1]

(1) Universidad San Sebastián, School of Medical Technology, Medicine and Science Faculty, Santiago, Chile

Background. Maternal physiological hypercholesterolemia (MPH; total cholesterol (TC)≤280 mg/dL) occurs during pregnancy to satisfy fetus requirements. However, some women develop maternal supraphysiological hypercholesterolemia (MSPH; TC>280 mg/dL), which associates with detrimental consequences on maternal and fetoplacental vascular health, such as endothelial dysfunction1,2. During pregnancy, the placenta secretes several soluble mediators, such as small extracellular vesicles (sEVs)3, which could be modified by maternal hypercholesterolemia and could modulate their vascular health by mediating pro-atherogenic effects on endothelial cells. Aim: To characterize the biology and function of placental-derived sEVs from MPH and MSPH pregnant women on endothelial cells. Methods. sEVs from term MPH (n=4) and MSPH (n=4) placental explant cultures (ethics approval #180810004, Pontificia Universidad Católica de Chile) were purified by differential ultracentrifugation. sEVs were characterized and quantified by nanoparticle tracking analysis (NTA), transmission electron microscopy, sEVs markers, and protein and cholesterol content. sEVs function was analyzed in endothelial cells (HMEC-1) by determining its effects on endothelial activation, intracellular nitric oxide (NO) levels, and endothelial NO synthase (eNOS) protein expression. Values are mean ± standard error mean. Data were analyzed by t-test and considered significant when $p<0.05$. Results. In MSPH, placental-derived sEVs concentration was higher than in MPH women, without changes in the mode size of the particles or their morphology. The protein concentration was higher in MSPH-sEVs compared to MPH-sEVs, without changes in their cholesterol content. Endothelial cells incubated with sEVs derived from MSPH placentas showed a reduced protein expression and activity of eNOS, compared to sEVs from MPH placentas, without changes in endothelial activation. Conclusion. MSPH associates with changes in the concentration and function of placental-derived sEVs, which could contribute to MSPH-associated endothelial dysfunction and the pathogenesis of maternal hypercholesterolemia during pregnancy by exerting pro-atherogenic effects on relevant cells for vascular health as endothelial cells.

Keywords: Maternal supraphysiological hypercholesterolemia, Small extracellular vesicles, Endothelial cells

Financing: FONDECYT Regular 1230527. Proyecto VRID_PDOC22/01 (USS). This work had the support of the "Vicerrectoría de Investigación y Doctorados" of the San Sebastián University – Project VRID_PDOC22/01.

Conditioned cell culture medium from stressed BeWo cells induces activation of HMEC-1 cells.

Ivo Carrasco-Wong[1], Anyelo Durán[1], Catalina Alvarado-Mancilla[1], Delia Indira Chiarello[1], Jaime Guitérrez[1]

(1) Cellular Signaling and Differentiation Laboratory (CSDL), School of Medical Technology, Health Sciences Faculty, Universidad San Sebastian, 7510156 Santiago

Background. Early-onset preeclampsia (EOPE) is a severe syndrome of pregnancy. The etiology is a deficient remodeling of the uterine arteries, which maintains a low blood flow and high pressure into placenta. Mothers with EOPE has an endothelial dysfunction associated to chronical inflammatory state(1). It is currently unknown how this inflammation is initiated and maintained during pregnancy. We propose that stressed syncytiotrophoblast is a crucial player in this phenomenon by the releasing of an altered secretome. This report shows the results of our proof-of-concept. Methods. Third pregnancy trimester cytotrophoblas cell line (BeWo) was induced to fuse or not with 20 μM of forskolin or 0,1% DMSO, respectively, for 48h. Then, cells were exposed to a preeclampsia in vitro model, an initial incubation at 1% O2 for 16h (Hypoxia) followed by 20% O2 for 8h (Reoxygenation), H/R-protocol(2). for normoxia control groups, DMSO- and FSK- BeWo were incubated at 20% O2 for 24h, NX-protocol. Conditioned cell culture mediums were collected and used as “treatment” for 24h on HMEC-1, microvascular endothelial cell line, in a 30% (v/v) proportion. VCAM-1 and ICAM protein level was measured by Western blot. Also, HMOX-1, SOD2, IL-8 and IL-6 mRNA accumulation was measured by RT-qPCR. Results. VCAM-1 and ICAM protein levels were increased in FSK-NX and DMSO-H/R, compared to DMSO-NX. Between H/R groups, VCAM-1 was elevated in FSK-H/R. The SOD2 mRNA level was increased in FSK-H/R, compared to DMSO-H/R. The levels of IL-6 mRNA were elevated in FSK-H/R compared to DMSO-H/R and to FSK-NX. Also, FSK-NX showed higher levels of IL-6 compared to DMSO-NX. Conclusions. The secretome of BeWo induced to fuse, in hypoxia/reoxygenation, can induce activation of HMEC-1, incrementing classical adhesion proteins and the IL-6 mRNA expression. The SOD2 mRNA accumulation suggests the induction also of oxidative stress.

Keywords: Syncytiotrophoblast-stress, secretome, endothelial-activation

Financing: PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2021, Folio N°SA77210087 to IC-W,

Acknowledgments: PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2019, Folio N°PAI77190060 to DC., FONDECYT 1221362 to JG (Universidad San Sebastián, Chile). CONICYT.

Use of cell trackers for the proper determination of BeWo cell fusion.

Catalina Alvarado-Mancilla[1], Javiera M. Sánchez[1], Ányelo Durán[1], **Ivo Carrasco-Wong[1]**, Pascuala Valdivia[1], Jaime Gutierrez[1]

(1) Cellular Signaling and Differentiation Laboratory, School of Medical Technology, Medicine and Science Faculty, Universidad San Sebastian, 7510156 Santiago, Chile.

Background. Syncytiotrophoblast (STB) is a fundamental specialized cell network in the placenta formed by the fusion of cytotrophoblast cells (CTB). BeWo cells, a human cytotrophoblast cell line, can be induced to syncialize by forskolin (FSK) treatment, secreting human chorionic gonadotropin (hCG) 1, a marker of a functional syncytiotrophobalst. However, the evaluation of the fusion process, commonly based on the determination of the number of nuclei delimited by plasma membrane, results to be costly and time consuming, since required immunofluorescence staining. Here we propose the use of fluorescent dyes for a rapid and simple method to evaluate the BeWo fusion process and possibly other cell types. Methods. To evaluate cell fusion, green and red fluorescent dye (Celltrackers) CMFDA and CMTPX, respectively (10 μM) were used to stain two groups of BeWo cells for 30 min. Then, equal amount of each group was mixed and seeded on covers and treated with 20 μM FSK or 0,1% DMSO for 48 or 72 h, in standard BeWo culture conditions. To evaluate syncytiotrophoblast commitment the cells were fixed in PFA 4% and stained whit anti hCG antibody (far red) and then analyzed in a TCS SP8 Leica confocal microscope. Additionally, RT-qPCR analysis was made to evaluate mRNA levels of Syncitin-1 and Syncitin-2, two markers of cell fusion. Results. Separated groups of green and red cells were observed in DMSO treatment at 48 and 72 h, with no expression of hCG. Contrary, cells treated with FSK (48 and 72h) showed clearly fused cells evidenced by the colocalization of green and red tracker. This fused cells also showed a high expression of hCG. The mRNA accumulation of Syncitin-1 and -2 was elevated at 48 and 72h. Conclusion. The staining of BeWo with different cells trackers is a rapid and simple method to evaluate cell fusion.

Keywords: bewo, cell-tracker, fusion

Financing: PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2021, Folio N°SA77210087 to IC-W

Acknowledgments: FONDECYT 1221362 to JG (Universidad San Sebastián, Chile). CONICYT.

Hippocampal lesions not only affect memory and learning but also reproductive functions.

Estrella Del Mar Castillo López[1,3], Andrea Coba de Ita[1], Daniel Vazquez Piñan[1], Yarey Calixto Maldonado[1], Lorena Milflores Flores[3], Rosalina Reyes Luna[4], Gonzalo Flores Álvarez[2], Ubaldo Quiróz Lopez[1]

(1) Facultad de Ciencias Biológicas, (2) Laboratorio de Neuropsiquiatría, Instituto de Fisiología, (3) Laboratorio de Biología Molecular, Facultad de Ciencias Biológicas, (4) Laboratorio de Biología de la reproducción, Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, Puebla, México

Background. Ovarian functions are regulated by the hypothalamic-pituitary-gonadal axis and by a direct neural pathway between the central nervous system and the ovaries. The hippocampus is not yet considered to participate in the regulation of reproduction; however, various studies have shown that in Huntington's disease, cognitive dysfunctions are observed that are associated with neurodegeneration of the hippocampus, interestingly, gonadal dysfunctions are also present. The aim of this study was to analyze the effects of dorsal or ventral hippocampal lesion in female rats on dendritic spine density of hippocampal pyramidal neurons in CA1 and CA3 areas, onset of puberty, and follicular growth.

Methods. Prepubertal female rats of the Long-Evans strain of 21 days of age were used. Ventral or dorsal hippocampal lesions were performed by stereotaxic surgery as were their respective controls, a fifth group was used as absolute control. At 30 days of age, all groups underwent novel object recognition (NOR) tests. The age at which they presented vaginal opening was recorded and they were sacrificed at the first vaginal estrus. All the procedures described were approved by the Internal Committee for the care and use of laboratory animals (CICUAL-100519168-UALVIEP-22/1).

Results. The results of the NOR memory test showed a decrease in short-term and long-term memory in the animals of the groups with lesion of the ventral or dorsal hippocampus in comparison with their respective controls and the absolute control group. Similarly, they presented a significant decrease in the density of dendritic spines in the CA1 region compared to the CA3 region, a delay in the age of vaginal opening, a decrease in the number of ovarian follicles and an increase in atresia. These results support the idea that the hippocampus participates not only in memory and learning processes, but also in the regulation of gonadal functions and reproduction.

Keywords: Hippocampus, dendritic spines, ovarian follicles

Financing: Funding. CONACyT (005671), CONCYTEP and VIEP.

Evaluation of Dietary Intake among Pregnant Women Attended in a Public Health Center in Chile: A Preliminary Study

A Bertini[1,2,3,4,5], F Fuentes[1,2,3], V Palma[6], A Rodriguez[6], M Pino[7], J Moya[8], J Gutierrez[9], **DI Chiarello[9]**, F Pardo[1,2,3,10]

(1) Metabolic Diseases Research Laboratory (MDRL), (2) Interdisciplinary Center for Research in Territorial Health of the Aconcagua Valley (CIISTe Aconcagua), (3) Biomedical Research Center and (4) Programa de Doctorado en Ciencias e Ingeniería para la Salud, Universidad de Valparaíso, San Felipe, Chile. (5) Millennium Institute for Intelligent Healthcare Engineering, Santiago, Chile. (6) Universidad San Sebastián (USS), School of Obstetrics, Faculty of Health Care Sciences, Santiago, Chile. (7) Universidad de las Américas, School of Obstetrics and Childcare, Faculty of Health and Social Sciences, Santiago, Chile. (8) USS, School of Nutrition and Dietetics, Faculty of Health Care Sciences, Santiago, Chile. (9) USS, School of Medical Technology, Faculty of Medicine and Science, Santiago, Chile. (10) Universidad de Valparaíso, School of Medicine, San Felipe, Chile

Background. The diet during pregnancy must be healthy and nutritious for an optimal pregnancy without complications. For this reason, it was decided to evaluate the diet of pregnant women who attended the public health center in Chile. Methods. After approval of the project by the ethics committee, dietary intake was evaluated by applying two 24-hour reminders of the previous day (R24) in pregnant women in their first trimester (15 pregnant women). The questionnaire was for each pregnant woman on two days of a conventional week. The SER24H software of the Instituto Tecnológico de los Alimentos of the Universidad de Chile was used to quantify macronutrients and micronutrients. The final database was analyzed in IBM SPSS Statistics 25 software. Results. The mean age of the pregnant women who attended was 29 ± 5 years, and their mean BMI was 30±5 kg/m2 (13.3% normal weight, 40% overweight, 46.7% obese). The average caloric intake of pregnant women in their first trimester was 1,527 ± 595 calories. According to the RDA guideline, it was found that 46.7% of pregnant women had an excessive intake of carbohydrates, while 66.7% and 73.3% had insufficient intake of protein and fiber, respectively. According to our findings, inadequate intake was observed among pregnant women concerning calcium (60%), zinc (73%), and folate (90%). Additionally, all pregnant women (100%) had insufficient iron intake. Conclusion. These findings highlight the importance of addressing nutritional deficiencies and imbalances in pregnant women's diets. These results are preliminary; we intend to work in a multicenter manner and increase the number of participants to validate the analyses. The results are exclusively the contribution of nutrients from the diet and do not include the analysis of food supplements.

Financing: FONDEF/XVIII Concurso Nacional de Proyectos de Investigación y Desarrollo en Salud, FONIS-7698SA21I0099 and ANID-PhD scholarship 21231727 (to AB)

Assessing compliance to recommendations on dietary patterns in pregnant women who attended Public Health Service during 2022-2023 in Valparaiso and Metropolitan Region, Chile.

A Bertini[1,2,3,4,5], F Fuentes[1,2,3], A Rodriguez[3,6], M Pino[7], V Palma[6], J Moya[8], J Gutierrez[9], F Pardo[1,2,3,10], **DI Chiarello[9]**

(1) Universidad de Valparaíso (UV), Metabolic Diseases Research Laboratory (MDRL), S. Felipe, Chile. (2) UV, Interdisciplinary Center for Research in Territorial Health of the Aconcagua Valley, S. Felipe, Chile. (3) UV, Biomedical Research Center, S. Felipe, Chile. (4) UV, Programa de Doctorado en Cs. e Ingeniería para la Salud, Valparaíso, Chile. (5) Millennium Institute for Intelligent Healthcare Engineering, Santiago, Chile. (6) Universidad San Sebastián (USS), School of Obstetrics, Faculty of Health Care Sciences, Santiago, Chile. (7) Universidad de las Américas, School of Obstetrics and Childcare, Faculty of Health and Social Sciences, Santiago, Chile. (8) USS, School of Nutrition and Dietetics, Faculty of Health Care Sciences, Santiago, Chile. (9) USS, School of Medical Technology, Faculty of Medicine and Science, Santiago, Chile. (10) UV, School of Medicine, S. Felipe, Chile

Background. A balanced and diverse diet is important for optimal gestational weight gain. We analyzed the dietary patterns in pregnant women according to Chilean ministerial indications. Methods. Part of the data are from the mami-educ randomized clinical trial (RCT) (ClinicalTrials.gov:NCT05114174). After approval by the ethics committee, the Questionnaire of Feeding Patterns in Pregnant Women (CPAG) was applied virtually to 89 pregnant women during the 1st trimester attending public service in the Metropolitan and Valparaíso Regions in Chile. This questionnaire consists of 14 multiple-choice questions. The data were analyzed in IBM SPSS Statistics 25. The variables were analyzed according to the percentage of compliance with the ministerial recommendation. Results. In the 1st of pregnancy, 41.6% adequately met the recommended intake of vegetables (consuming ≥2 servings/day), while 59.6% reported sufficiently consuming fruits (≥2 daily servings). Regarding dairy products, 50.5% consumed ≥2 daily servings. Only 24.7% and 34.8% of fish and legume intake met the recommendation of ≥2 weekly servings. Only 38.2% complied with the recommendation of consuming six or more glasses of water per day. Regarding unhealthy food choices, 30.3% rarely or never consuming foods with warning seals. Furthermore, 83.2% reported not using salt for seasoning, while 51.7% rarely or never consumed sugared beverages. As for fried foods, 66.3% reported consuming them less than 1-2 times a month. Conclusion. Pregnant women show a low consumption of fruits and vegetables, fish, legumes and water. However, they avoid the use of salt. Increased promotion of healthier eating habits during pregnancy is needed.

Financing: FONDEF/XVIII Concurso Nacional de Proyectos de Investigación y Desarrollo en Salud, FONIS-7698SA21I0099 and ANID-PhD scholarship 21231727 (to AB)

Revisiting weight gain during pregnancy: Maternal lipid levels association with body mass index, and excessive weight gain at term of pregnancy. High lipid levels during postpartum.

Susana Contreras-Duarte[1], Daniela Moya[2], Ricardo Parra[3], Marcelo Farías[4], Andrea Leiva[5]

(1) Universidad San Sebastián, Faculty of Health Care Sciences, Santiago, Chile. (2) Pontificia Universidad Católica de Chile, Faculty of Nutrition and Metabolism, Santiago, Chile. (3) European Southern Observatory, Paranal Optics Group, MSE, Paranal, Chile. (4) Pontificia Universidad Católica de Chile, Faculty of Obstetrics and Gynecology, Santiago Chile. (5) Universidad San Sebastián, Faculty of Medicine and Sciences, Santiago, Chile

Background: Maternal weight gain (WG) during pregnancy is controlled to avoid gestational diseases and health consequences for the dyad. During gestation is recommended increase weight according to the nutritional stage at the beginning of pregnancy. However, it is unknown whether the excessive weight gain (EWG) could affect maternal lipid levels during gestation, and if during postpartum period (PP) lipids are controlled. Methods: Clinical information was obtained from a database of 1055 women. Ethics approval number 210120006 from the Pontificia Universidad Católica de Chile committee. Women were categorized according to gestational age in first (T1), second (T2), and third trimester (T3) of gestation. PP period was considered until 12 months after delivery. Women were categorized according to BMI at the start of pregnancy with normal (NW), overweight (OW), and obesity (OB). WG was calculated as kilograms (kg) gained from T1 to T3. EWG was considered >16 kg for NW, >11kg for OW, and >9kg for OB. Lipids levels were determined by enzymatic-colorimetric assays. Dyslipidemia was considered as TC levels >291, LDL >169, and TG >275 at T3 (units are mg/dL). Correlation and Regression models were performed between lipids and BMI or WG. Results: Total population presented increased LDL-levels from T1 to PP, high TC and TG-levels from T2 to PP, and decreased HDL in T2 and T3 period, respect to PG period. Negative correlations were found between TC-levels at T3 and BMI during T1; TC-levels at T3 and BMI during T3; LDL-levels at T3 and BMI during T3. While positive correlations were among WG and TC, LDL and HDL-levels at T3 period. EWG was positively correlated with TC at T3. Importantly, associations were observed between BMI at T1 and T3 with TC and LDL-levels at T3, and WG with TC and LDL-levels at T3. Conclusions: Lipid alteration associates with WG during pregnancy.

Keywords: excessive weight gain, lipid levels, pregnancy, postpartum, body mass index

Financing: Subvención a la instalación en la academia SA77210098, FONDECYT Regular 1230527.

Acknowledgments:
References10.1097/01.AOG.0000437382.03963.8810.1067/mob.2001.10959110.1067/mob.2001.109591

Maternal platelet secretome regulates the migration/invasion and endothelial-like transition of extravillus trophoblast: a research proposal.

Rodrigo Escalona[1], Jaime Gutiérrez[1]

(1) Universidad San Sebastian, School of Medical Technology, Faculty of Medicine and Science, Lota 2465, Providencia, Santiago, Chile

Background. In early human gestation, extravillous trophoblasts (EVTs) must invade the maternal decidua to remodel the uterine spiral arteries. Although a plug of trophoblasts occludes the lumen of the maternal spiral arteries, preventing the premature passage of erythrocytes and leukocytes to the intervillous space, but platelets can pass. Therefore, maternal platelets are considered the first maternal blood cells in the intervillous space. Morphological alterations and platelet dysfunction have been described in women with preeclampsia (PE), even before the onset of clinical manifestation, suggesting that they play a role in placental development and the etiology of PE. We propose that dysfunctional platelets of the mother, associated to an altered platelet-secretoma (PLTsec), could alter the early development of the placenta, generating a predisposition to have PE. Methods. Due to the difficulty of not being able to anticipate a pregnancy, much less know if it will present PE or not, we plan to obtain the PLTsec from women who have had normal pregnancies or PE in the past, even years after their pregnancy. We plan to use the PLTsec to study migration/invasion and mesenchymal to endothelial-like transition (MELT) ability in HTR8/SVneo, a first trimester human EVTs cell line. Expected Results. We expect that the PLTsec of women with PE pregnancies will increase the migratory capacity of HTR8/SVneo compared to normal pregnancies. In addition, we expect that decrease MELT in HTR8/SVneo, which is related to a decrease in endothelial markers. Projections. Platelets would have a role in the early development of the placenta, an alteration in its secretome could contribute to a dysfunction in the EVTs. We hope to be able to access to samples from the 1st, 2nd and 3rd trimester of healthy pregnant women who will generate PE to study the PLTsec and its possible participation in PE.

Keywords: Placental development, preeclampsia, platelet function

Financing: FONDECYT Regular 1221362.

"Phenotypic characterization of patients with polycystic ovarian syndrome from a population of the Ecuadorian Andes".

María Elena Espinosa[1,2], Estefanía Bautista-Valarezo[2], Stephanie Cristina Aguiar Hidalgo[2], Tamara Otzen[1], Carlos Manterola[1], Raúl Sánchez[3]

(1) Universidad de La Frontera, Programa de Doctorado en Ciencias Médicas, Temuco, Chile. (2) Universidad Técnica Particular de Loja, Facultad de Ciencias Médicas, Loja, Ecuador. (3) Universidad de La Frontera, Center of Excellence in Translational Medicine-Scientific and Technological Bioresource Nucleus (CEMT –BIOREN), Temuco, Chile

Introduction: Polycystic ovarian syndrome (PCOS) is one of the most common causes of menstrual cycle disorders, female infertility and hyperandrogenism. Its etiology is multifactorial and involves genetic, epigenetic and environmental factors. It is also accompanied by metabolic alterations such as hyperinsulinemia, insulin resistance, dyslipidemia and obesity. Objective: To determine PCOS phenotypes in women from the Ecuadorian Andes and to study their association with clinical, biodemographic, metabolic, and reproductive variables. Method: A cross-sectional study was carried out between January 2022-March 2023, to women of reproductive age diagnosed with PCOS. Biodemographic data and blood samples were obtained to analyze clinical, metabolic, and reproductive variables. The diagnosis of PCOS was made applying the Rotterdam criteria. In the statistical analysis, the qualitative variables were associated, obtaining the OR and chi square. Results: The participants were 68 women of reproductive age between 18 and 40 years old, inhabitants of the city of Loja, who attended a gynecological and endocrinological consultation at the Hospital Santa Inés and were diagnosed with PCOS. Average age 22 years, 97% mestizo origin, 94.1% urban origin, 95.6% single, 95.6% higher educational level and 51.6% medium-high socioeconomic level. Phenotype A corresponds to 63%, Phenotype B 12%, Phenotype C 13% and Phenotype D 12%. The A and B phenotype is the one that is predominantly associated with clinical (oligomenorrhea-amenorrhea), metabolic (direct bilirubin) and reproductive (17OH progesterone) variables with statistically significant values (p value: < 0.05). Phenotypes C and D did not show association with any variable. Conclusions: The Rotterdam Consensus considers different phenotypes in the same syndrome, which could represent different degrees of severity of the same disease. This situation was also apparent in our study. In addition, it can be concluded that despite the high range of tests carried out, the statistically significant variables were few. Ethical Statements: Ethics Committee of the U. of Cuenca # 2022-002EO-IE

Keywords: Polycystic ovary syndrome, phenotypes, hyperandrogenism, metabolism

Financing: no external funding received. Acknowledgments: Department of Imaging and laboratory of the Hospital Santa Inés Loja.

Characterization of DRD2 and DRD3 dopamine receptors in placentas from normal and severe preeclampsia pregnancies

Isabel Gómez[1,2], Rodrigo Pacheco[3], Jaime Gutiérrez[2]

(1) Universidad San Sebastián, PhD program in Immunology and Microbiology, Fundación Ciencia & Vida, Medicine and Science, Lota 2465 Providencia, Santiago, Chile. (2) Universidad San Sebastián, Cellular Signaling and Differentiation Laboratory, School of Medical Technology, Medicine and Science, Lota 2465 Providencia, Santiago, Chile. (3) Universidad San Sebastián, Laboratory of Neuroimmunology, Fundación Ciencia & Vida, Medicine and Science, Avenida del Valle Norte 725 Huechuraba, Santiago, Chile

Background: Severe preeclampsia (sPE) is a hypertensive disorder that affects pregnant women, associated with fetal-maternal morbi-mortality. Its pathophysiology involves deficient invasion and endothelial-like differentiation of trophoblast cells, associated to abnormal placental development, impaired uteroplacental circulation and maternal immunological imbalance (1). Dopamine is a neurotransmitter that regulates cell invasion (2), vascular tone, blood pressure and immune balance (3). However, its role in placental and sPE development has not been described. The aims of this study were to characterize the expression and distribution of dopamine receptors DRD2 and DRD3 in placentas of mother with sPE or control pregnancies, and its role as a regulator of trophoblast invasion and differentiation. Methods: Samples from normal and sPE pregnancies were collected. qPCR and western blot analysis were performed to quantify the levels of DRD2 and DRD3 in placental extracts. Immunofluorescence staining was conducted to observe the receptor's localization and distribution. Invasion and endothelial differentiation assay were conducted in human first trimester trophoblast cell line to assess the role of dopamine. Results: Our findings indicate that DRD2 and DRD3 are expressed in placental samples (normal and sPE) with differences in the localization and intensity. In normal pregnancies, DRD2 appears to be localized at the apical side of syncytiotrophoblast membrane; DRD3 localized mainly in stromal and endothelial cells of placental villi. In sPE, DRD2 present a wider distribution on syncytiotrophoblast, mainly intracellular; a similar phenomenon occurred with DR3 expression, which increased in the latter group. Conclusions: Dopamine receptors are increased in sPE placentas showing an atypical distribution. Furthermore, dopamine decreases endothelial differentiation of trophoblast and as well as its invasion ability. These results suggest that the increase in the expression of DRD2 and DRD3 may be involved in the pathophysiology of PE. The study protocol was approved by the Scientific Ethics Committee of the Pontificia Universidad Católica de Chile (#210618011)

Keywords: Preeclampsia (PE), trophoblast, dopamine, Dopamine Receptor 2 (DRD2), Dopamine Receptor 3 (DRD3)

Financing: FONDECYT 1221362FONDECYT 1210013

What is known about the effect of SARS-CoV-2 and vaccines on ovarian function?

Yamila Herrero[1], Candela Velazquez[1], Mayra Bordaquievich[1], Melanie Neira[1], Vanesa Hauk[2], Ignacio De Zúñiga[3], Gustavo Martinez[4], Mariano Lavolpe[5], María Florencia Veiga[6], Leopoldina Scotti[7], Dalhia Abramovich[1], Fernanda Parborell[1]

(1) Institute of Experimental Biology and Medicine (IByME) – CONICET Buenos Aires, Argentina. (2) Institute of Biological Chemistry (IQUIBICEN), School of Exact and Natural Sciences, University of Buenos Aires-CONICET, Buenos Aires, Argentina. (3) Pregna Medicina Reproductiva, Buenos Aires, Argentina. (4) Fertilis, Medicina Reproductiva, Buenos Aires, Argentina. (5) In Vitro, Buenos Aires, Argentina. (6) We Fiv, Buenos Aires, Argentina. (7) Centro de Investigaciones y Transferencia del Noroeste de la Provincia de Buenos Aires, (CITNOBA-UNSADA-CONICET), San Antonio de Areco, Argentina

Background. We have previously shown that alterations in ovarian function can be observed up to 9 months after SARS-CoV-2 infection. So far, no studies have determined the long-term effects of SARS-CoV-2 infection and the vaccine on fertility. We propose two objectives: a) To evaluate ovarian function in patients who have suffered from COVID-19 (mild symptoms) up to 18 months post-infection and b) To assess the effect of vaccines against COVID-19 on reproductive performance. Methods. A total of 125 women (21–43 years old) under ART were recruited between November 2020 and May 2022. For objective a), the patients were classified into: control group (n=30) (no positive test for COVID-19) and the post-COVID-19 group (n=55) (at least one positive test by PCR). For objective b), patients are classified into: the unvaccinated group (n=70) and the group vaccinated for COVID-19 (n=55). This work complies with ethical requirements. Results. The results showed that in patients (without vaccination) the number of retrieved and mature oocytes decreased up to 12 months after COVID-19 infection compared to the control ($p<0.01$). When patients with and without vaccines are considered together, the number of retrieved and mature oocytes decreases even up to 18 months post-infection in those patients older than 35 years. Reduced levels of IL-1β in FF were observed in patients up to 18 months post-infection compared to the control group ($p<0.001$) without changes in IL-10 and VEGF levels. In endothelial cells stimulated with post-COVID-19 FF, we observed a decrease in cell migration without changes in VEGF and ANGPTs. The number of retrieved and mature oocytes is unchanged in vaccinated patients compared to unvaccinated patients regardless of the type of vaccine and infection. In conclusion, our results show that ovarian function remains altered up to 18 months after infection. Further, the vaccines do not affect female fertility.

Financing: This work was supported by FONCyT (PICT 2021 No.06), Ferring Pharmaceuticals.

Acknowledgments: Fundación IByME, Fundación René Barón, Fundación Williams

Machine learning analysis of near-infrared spectra reveals biochemical alterations in follicular fluid of endometriosis patients

Catalina Cortés-Stuardo[1], Valeska Jara-Riquelme[2], Jorge Bardisa[2], Italo Ciuffardi[2], Juan Araya[3,4], Enrique Guzmán-Gutiérrez[1,4], **Daniela Mennickent[1,3,4]**

(1) Universidad de Concepción, Departmento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Concepción, Chile. (2) Instituto de Medicina Reproductiva, Concepción, Chile. (3) Universidad de Concepción, Departamento de Análisis Instrumental, Facultad de Farmacia, Concepción, Chile. (4) Machine Learning Applied in Biomedicine (MLAB), Concepción, Chile

Background. Endometriosis is a chronic inflammatory disease that affects 5-10% of women in reproductive age (1). Nearly 30-50% of endometriosis cases are linked to infertility (1). Moreover, this disease is associated with poor assisted reproductive technology (ART) outcomes, mainly because of a decreased number of oocyte retrieved and a reduced implantation rate (2). The biochemical mechanisms underlying the pathophysiology of endometriosis, and its relationship with both infertility and impaired ART success are not fully understood (3). The analysis of follicular fluid (FF) (4) with vibrational spectroscopy and machine learning (ML) (5) could help reveal such mechanisms. This study aims to compare FF samples from women with and without endometriosis, using near-infrared (NIR) spectroscopy and ML. Methods. This work was approved by Instituto de Medicina Reproductiva (IMR) and was carried out considering the Declaration of Helsinki. FF was collected by transvaginal follicular aspiration, from women undergoing ART (n=7 for control, n=4 for endometriosis) at IMR, Concepción, Chile. 10 µl of each sample were deposited on a mirrIR plaque, and dried at 37ºC for 30 minutes. Three NIR spectra (10500-4000 cm-1) were acquired and averaged for each sample. Spectral data were pretreated by 2-norm normalization and mean centering, and then analyzed by the exploratory ML technique principal component analysis (PCA). Results. Endometriosis FF samples are completely separated from control FF samples by principal components 2 and 3 (PC2 and PC3). This separation is attributable to differential spectral patterns in wavenumber regions associated with proteins, lipids and carbohydrates. Conclusions. Several groups of biomolecules are altered in the FF of patients with endometriosis. The identification of particular proteins, lipids and carbohydrates with other analytical techniques could help to better understand the pathophysiological mechanisms of endometriosis, and its connection with infertility and ART failure.

Keywords: Infertility, Bioanalytical chemistry, Artificial intelligence

Financing: None. Acknowledgments: The authors would like to thank Joanna Tereszczuk from CMA Bio-Bío for her technical support.

Maternal Spotty factor controls meiotic chromosome segregation during the zebrafish oocyte-to-egg transition

Bárbara Palma[1,3], Ruth Cisternas[1,3], Constanza Aguirre-Campos[1,3], Felipe Aguilera[2,3], Fabián Segovia[1,3], Mary C Mullins[4], Ricardo Fuentes[1,3]

(1) Department of Cell Biology, (2) Department of Biochemistry and Molecular Biology and (3) Developmental Processes Group, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile. (4) Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.

Background. Oogenesis is the process by which the female gamete, or oocyte, is formed. During this process, it is essential for the chromosomes to be distributed accurately and equitably in the daughter cells to ensure proper embryo development. Chromosome segregation is regulated by maternal gene products, which play critical roles in oocyte development, egg activation and early embryogenesis. While some maternal genes involved in this process have been identified, many others remain undiscovered. This highlights the significance of uncovering new genes to gain a comprehensive understanding of oogenesis regulation. Methods. Using a forward genetic screen, we have isolated the maternal-effect spotty mutant whereby females produce eggs that activate development but fail to initiate the first cell division. By using cellular, molecular and bioinformatics approaches, and high-resolution imaging, we studied the mutant phenotype in whole ovaries, isolated oocytes, eggs and zygotes. Results. Phenotypic analysis revealed that spotty oocytes and eggs exhibit numerous MTOCs and aster-like structures after fertilization. Based on imaging analysis, we found that meiotic chromosome segregation and mitotic nuclear assembly appear affected in the mutant oocyte and egg, respectively, which have adverse consequences for mononuclear assembly after fertilization. Interestingly, cell-free cytoplasmic extracts analysis indicates that the spotty gene lesion generates changes in proteomic profiles of zebrafish oocytes. Conclusions. Studying the spotty mutant provides a valuable opportunity to unravel the molecular mechanisms governing reproductive traits under its regulation. Our mutant analysis revealed that the maternal Spotty factor represses the formation and nucleating activity of MTOCs, a critical process involved in ensuring the correct distribution of chromosomes during meiosis. Additionally, DNA integrity maintenance in the oocyte might be regulated by a stage-specific dosage of products from maternal genes. These findings provide knowledge in advancing our understanding of reproductive development, causes of chromosomal anomalies, and developmental defects in vertebrate organisms, including humans.

Keywords: Oogenesis, Zebrafish, Meiotic Chromosome Segregation

Financing: FONDECYT de Iniciación 11201118 Project

Post-insemination endometritis increases heat shock protein 90 immunoexpression in mares

Ana Laura Camacho[1], Paula Lombide[1], María Helen Viotti[1], Nicolás Cazales[2], Graeme B Martin[3], **Graciela Pedrana[1]**

(1) Universidad de la República, Biociencias, Veternaria, Ruta 8 Km 18, Montevideo, Uruguay. (2) Universidad de la República, Producción Animal, Veterinaria, Route 8, Km 18, Montevideo, Uruguay. (3) University of Western Australia, Perth, Australia

Background. Post-breeding endometritis in mares is a physiological response of the endometrium to seminal plasma and must be prevented to avoid gestational losses[1]. Heat shock proteins (HSPs) could be involved because they play roles in inflammation and protein-folding in uterine tissue in women[2] and in healthy mares[3]. We tested whether HSP90 expression is increased by endometritis in equine endometrium. Methods. The experiment was approved by Commission for Animal Experimentation Nº 111130-001637-13. Endometrial biopsies were obtained in a commercial equine slaughterhouse, Canelones, Uruguay from Creole mares randomly assigned to a Control (in oestrus; n = 8) or after 2 (n = 8) or 4 hours (n = 8) post- artificial insemination. HSP90 immunostaining area (%) was measured in the epithelium, glands, and endometrial stroma in biopies by immunohistochemistry. Data were analyzed with ANOVA. Results. Immunoexpression of HSP90 was intense in cell nuclei in the epithelium, glands and in stromal cells. The area of HSP90 immunoexpression was greater at 4 hours (6.5 ± 0.35%) and 2 hours post-insemination (5.0 ± 0.4%) than in the Control (1.9 ± 0.4%; P = 0.0001). Conclusions. Post-insemination endometritis induced an increase in HSP90 within the cell nuclei in endometrial tissues. The nuclear location is consistent with transcriptional regulation of HSP90, where the chromatin is opened at the promoter region of the HSP90 gene[4], allowing the action of sequence-specific transcription factors, such as HSF-1, and other factors as RNA polymerase[5]. An increase in nuclear HSP90 during endometritis suggests the up-regulation of genes that are essential for the initiation and maintenance of an inflammatory response.
References. 1. Canisso et al. (2020). Int J Mol Sci. 21(4):1432. 2. Jee et al. (2021). Front. Cell Dev. Biol. 9: 648463. 3. Camacho et al. (2021). Anat. Histol. Embryol. 50 (1): 50-57. 4.Su et al. (2016). Anticancer Res. 36(5):2197-203. 5. Calderwood and Neckers. (2016). Adv Cancer Res. 129:89-106.

Keywords: Endometritis, horse, uterus, HSP90

Financing: Comisión Sectorial de Investigación Científica (CSIC), Régimen Dedicación Total, Graciela Pedrana. Programa de Iniciación a la Investigación (CSIC), Universidad de la República, Uruguay, Ana Camacho. Acknowledgments: To Victoria Yozzi and Silvana Soria for her help in the histological procedures in the lab.

Effect of endometritis on blood flow and assessed by Doppler ultrasound

Ester Sabrina Castro[2], Paula Lombide[3], María Helen Viotti[3], Nadia Diugyz[2], Florencia Pereyra[2], María José Estradé[1], Rodrigo Mattos[4], **Graciela Pedrana[3]**

(1) Universidad de la República, Departamento de Producción Animal, Facultad de Veterinaria, Montevideo, Uruguay. (2) Universidad de la República, Departamento de Hospital - Unidad de Clínica y Cirugía de Equinos, Facultad de Veterinaria, Montevideo, Uruguay. (3) Universidad de la República, Biociencias, Facultad de Veterinaria, Montevideo, Uruguay. (4) Universidade Federal do Rio Grande do Sul, Reprodução, Reprolab, Faculdade de Veterinária UFRGS, Porto Alegre, Brasil

Background. Mating in mares produces an inflammation of the endometrium as a physiological response[1]. Blood flow plays a key role in inflammation, and vasodilation is one of the most important features of all inflammatory processes that include endometrial edema and leukocyte extravasation[3]. We hypothesized that induced endometritis will increase blood flow in the uterus. Hence, we select a non-invasive color Doppler, B-mode ultrasonography technique that allows us to study blood flow in the uterus[4]. Methods. The experiment designed was approved by the Honorary Commission for Animal Experimentation (Nº 591/18). Mares in oestrus were assigned to a control group (n=9) or treated group (n=13, inoculated with Streptococcus equii zooepidemicus by intrauterine infusion with 1x109 in 20 ml of 0.9 NaCl in distilled water solution. Ultrasonographic examinations were performed in B mode for endometrial edema analysis and color Doppler mode for uterus blood flow evaluation. Data were analyzed with ANOVA. Results. The vascular area in the ipsilateral horn increased in the treated group compared to the control group (6.2 ± 0.3 vs 5.3 ± 0.3 %, P=0.03). A tendency to increase the percentage of vascular area was detected in the contralateral uterine horn (6.2 ± 0.3 vs 5.2 ± 0.4 %, P=0.07). Conclusions. Induced endometritis increased blood flow to the ipsilateral and a tendency to increase in contralateral horn. Our results agree with similar studies in the uterus of mares with endometritis that detected an increase in endometrial blood flow[5]. The quantitative image analysis determines endometritis in mares allowing an early diagnosis of endometritis through blood flow analysis.

References. 1. Köhne et al. (2020). 2. Debertolis et al. (2016). 3. Schnoor et al. (2021). 4. Guido et al. (2020). 5. Da Silva-Álvarez et al. (2022).

Keywords: Horse, uterus, doppler, endometritis, inflammation, Image, analysis

Financing: Comisión Sectorial de Investigación Científica (CSIC), Programa Proyectos I+D 2020, Beca Posgrados Nacional Agencia Nacional de Investigación e Innovación (ANII) María José Estradé.

Maternal Osbpl7 factor controls cleavage furrow formation during the embryonic first cell division

Ingrid Pinto Borguero[1,3], Natalia Benavente Cabrera[1,3], Felipe Aguilera[2,3], Mary C. Mullins[4], Ricardo Fuentes[1,3]

(1) Department of Cell Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile. (2) Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Universidad de Concepción, Chile. (3) Developmental Processes Group (GdeP), Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile. (4) Department of Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA.

Background: Early embryo development relies on maternally provided gene products loaded into the egg during oogenesis. One of the crucial processes controlled by this by this maternal contribution is the establishment of the first cell division after fertilization, which would give rise to a viable embryo. However, only a limited number of maternal regulators of this process have been identified to date. In addition, although membrane arrangements occur during cell division, little is known about specific roles of maternally-loaded molecules participating in lipid transport and recruitment during its execution. Methods: We have isolated the zebrafish osbpl7sa6256 mutant from a phenome-wide screen. We have also generated and analyzed a transcriptomic profile from unfertilized wild-type and mutant eggs. Furthermore, we performed immunostaining on eggs, zygotes, and early embryos, to examine the meiosis-to-mitosis transition, egg activation and cleavage furrow formation. By using bioinformatic tools, we studied the molecular phenotype of the maternal Osbpl7 factor. Results: Phenotypically, the mutant embryo resumed meiosis but failed to form the cleavage furrow during the first and subsequent cell divisions. In addition, egg activation appeared affected in the osbpl7 mutant. Remarkably, the mutant early embryo underwent lysis, indicating that the mutation is highly penetrant and lethal. A list of differentially expressed genes showed dysregulation of factors regulating microtubule stability, and lipid homeostasis. Structurally, the predicted maternal mutant protein indicated that it lacks part of the functional domain at the C-terminus, which transport oxysterol. Conclusions: Our findings establish, for the first time, a link between an oxysterol transporter and cell division during embryonic development, as well as reproductive success. Specifically, we have identified a crucial factor controlling the proper formation of the cleavage furrow during the embryonic first cell division.

Keywords: Osbpl7, Zebrafish, Lipid transport

Financing: ANID Doctorado Nacional 21221268, Fondecyt de Iniciación 11201118

Periodontitis-derived extracellular vesicles distribution on fetal-maternal tissues and adverse pregnancy outcomes occurrence in an in vivo model.

Carolina Rojas[1,2], María Luisa Mizgier[1], María José Bendek[1], Dolores Busso[3,4], Katina Schinnerling[5], Alejandra Chaparro[1]

(1) Universidad de los Andes, Centre for Innovation in Biomedical Research (CiiB), Faculty of Dentistry, Santiago, Chile. (2) Universidad de Chile, Faculty of Dentistry, Santiago, Chile. (3) Universidad de los Andes, Laboratory of Biology of Reproduction, CiiB, Faculty of Medicine, Santiago, Chile. (4) Universidad de los Andes, Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Santiago, Chile. (5) Universidad Andrés Bello, Facultad de Ciencias de la Vida, Santiago, Chile

Background. Periodontitis (Perio) is a chronic inflammatory disease triggered by a dysbiotic subgingival microbiota. Its infectious and immuno-inflammatory nature induces low-grade systemic inflammation, affecting several conditions. During pregnancy, Perio has been associated with the development of adverse pregnancy outcomes (APOs), such as preterm birth, low birth weight, and pre-eclampsia. Nevertheless, the precise mechanisms that explain these associations remain elusive. Perio-derived extracellular vesicles (Perio-EVs) are nano-sized particles released from the host immune-inflammatory and resident cells in response to microenvironmental queues, including outer membrane vesicles (OMVs) from periodontal bacteria. During Perio, the ulcerated and permeable gingival epithelium allows the translocation of Perio-EVs, into the tissue and bloodstream, endowing them the potential to disseminate. In the present study, we aimed to evaluate the systemic biodistribution of Perio-EVs and their effect on APOs development in pregnant periodontitis-affected mice. Methods. Perio-EVs were isolated from the gingival-crevicular fluid of Perio-affected patients and OMVs from the supernatant of Fusobacterium nucleatum bacterial cultures by ultracentrifugation. Perio-EVs or OMVs were stained with the fluorescent dye DiR and 2 x 108 EVs were inoculated intravenously into the tail vein of periodontitis-affected pregnant mice for 10 days. Biodistribution was evaluated in a Fluorescence Imaging System by assessing the presence of near-infrared signals within the different organs obtained. The evaluated APOs were fetus numbers, fetal weight and size, placental weight, fetal-placental ratio, craniocaudal distance, and the number of absorbed fetuses. All the experiments were carried out per the Guide for Care and Use of Laboratory Animals and approved by the University's Ethics Committee for Animal Welfare. Results. Perio-EVs systemically injected were detected in placentas and yolk sacs. Fetuses from Perio-affected pregnant mice exposed to Perio-EVs showed less weight, size, fetal-placental ratio, and craniocaudal distances than sham mice. Conclusions. Perio-EVs systemically injected reach the fetal-maternal unit in periodontitis-affected pregnant mice which may favor APOs development.

Financing: ANID CHILE FONDECYT REGULAR ID 1211471

Effects of oral contraceptives on metabolic parameters in adult premenopausal Chilean women

Matías Cabezas[1], Susan Calfunao[1], Nicolás Crisosto-King[2], **Francisca Salas-Pérez[3]**

(1) Laboratory of Chemical Carcinogenesis and Pharmacogenetics. Faculty of Medicine. Universidad de Chile. Santiago, Chile. (2) Laboratory of Endocrinology and Metabolism. Faculty of Medicine. Universidad de Chile. Santiago, Chile. (3) Health Sciences Institute. Universidad de O´Higgins. Rancagua, Chile.

Combined Oral Contraceptives (COCs) are the most used contraceptives in reproductive age women. COCs are combinations of low-dose estrogens and progestins. However, concerns have emerged regarding potential adverse cardiovascular and metabolic effects associated with COCs. COCs administration has been associated with the onset of glucose intolerance, hypertension, hypertriglyceridemia, and elevated CRP levels in healthy women. Objective: To evaluate the effect of combined oral contraceptives on parameters of lipid and carbohydrate metabolism in Chilean women. Methods: A total of 29 control women without hormonal contraception and 19 control women with hormonal contraception were included (BMI 18-35 kg/m²; 18-34 years old). To evaluate the metabolic impact, lipid and biochemical profiles were measured, and oral glucose tolerance tests were carried out. Student's t-test or Mann-Whitney test was applied to compare both groups. For all analyses, a p-value >0.05 was considered significant. The study protocol was approved by the Ethics Committee of Universidad de Chile (211-2020). Results: Women using oral contraceptive pills exhibited an increase in total cholesterol levels, along with elevated levels of HDLc, LDLc, and triglycerides. It is noteworthy to mention that the recruited women consumed different preparations, including progestins derived from testosterone, progesterone, and spironolactone. Additionally, a slight increase in HOMA-IR, a marker of insulin resistance, was detected in contraceptive users (1.75±1.1 vs 2.41±1.38,p-value: 0.041), independent of BMI. This increase was accompanied by a slight elevation in blood pressure levels (Systolic 112±11 vs 119±11;p-value: 0.029 and Diastolic 70.38±6 vs 76±8;p-value: 0.01). Conclusion: These results indicate that the use of combined oral contraceptives has small, yet potentially clinically significant effects on the metabolic profile of premenopausal women. To ensure a personalized clinical approach to oral contraception, it is crucial to conduct individualized risk stratification and management. This includes assessing each patient's personal cardiometabolic risk profile both at baseline and during follow-up, allowing for tailored care.

Financing: FONDECYT 11201337. Acknowledgments: Furthers collaborators: Dr Manuel Maliqueo, Bárbara Echiburú Members from the Laboratory of Endocrinology and Metabolism

The cellular distribution of RECK is regulated by its glycosylation's in human first trimester trophoblasts.

Lukas Salazar[1], Nicolas Daniel Alarcon Merino[1], Rodrigo Escalona[1], Jaime Gutierrez[1]

(1) Cellular Signalling and Differentiation Laboratory (CSDL), School of Medical Technology, Health Sciences, Lota 2465, 7510602 Providencia, Santiago, Chile

Background: Preeclampsia (PE), a severe pregnancy specific syndrome, is believed to originate from incomplete remodeling of uterine arteries due to insufficient invasion by extravillous trophoblasts. We found that the expression of RECK, a plasma membrane glycoprotein (GPI-anchored), which plays critical role as matrix metalloproteinases (MMP) and cell invasion inhibitor, is augmented in trophoblast cells from the severe form of PE. We found that RECK inhibit trophoblast invasion by a MMP dependent mechanism. By high-resolution SDS-PAGE and western blot analysis we found three bands of different molecular weights corresponding to RECK. One possible explanation is a differential glycosylation of the same protein. Which could also affect is cellular distribution and function. Objective: To determine the glycosylation of RECK and its potential role in defining its membrane localization in first trimester trophoblasts. Methods: RECK glycosylation and plasma membrane localization was analyzed in human first trimester cell line, HTR8/Svneo, by treating cell lysates with PNGase (removal of N-linked oligosaccharides from glycoproteins) or the treatment of cultured cells with tunicamycin (an inhibitor of N-glycosylation). Cell cultures were also treated with Phosphatidylinositol-specific phospholipase C (PIPLC), which release GPI-anchored proteins from the plasma membrane. Western blot of cell lysates and immunofluorescence analysis were performed. Results: PNGase treatment abolish the presence of higher, ~130kDa band, increasing at the same time, the intermediate, ~120kDa band. No changes in the lower, ~100 kDa band. Tunicamycin treatment, reduced the 130 kDa band without effects on the other (120 and 100 kDa bands). PIPLC treatment revealed that only the 130 and 100 kDa bands were forms of RECK present on the cell surface. Conclusions: RECK exhibits 3 bands of different molecular weights in HTR8/SVneo cells.

Keywords: Preeclampsia., RECK, glycosylation

Financing: FONDECYT regular 1221362.

Isolation and characterization of circulating placental extracellular vesicles using Tangential Flow-Filtration and Size Exclusion/Affinity Chromatography

Pascuala Valdivia[1,2], Delia Indira Chiarello[2], Jaime Gutiérrez[2]

(1) PhD Program in Cellular Biology and Biomedicine, Faculty of Medicine and Science, Universidad San Sebastián, Chile. (2) Cellular signaling and differentation Laboratory, Faculty of Medicine and Science, Universidad San Sebastián, Chile.

Introduction. Small extracellular vesicles (sEV) are particles released by cells into the extracellular environment contributing to intercellular communication. sEV have emerged as a tool for studying the physiological state of specific organs. Placental alkaline-phosphatase (PLAP), is a specific marker of placental derived sEV (sEV-PLAP+) which allows to purified this specific sEV from the maternal plasma, even from the early stage of pregnancy, allowing to monitor the process or to early diagnose pregnancy-related diseases. However, the isolation of pure sEV derived from plasma have been challenging due to the presence of high concentration of plasma proteins, poor yield, and questionable integrity, also the high cost, time consuming and expensive equipment. Our strategy aims to isolate sEV whit a high yield, purity, rapid, efficient and maintaining the integrity of the total sEV and sEV-PLAP+ from maternal plasma. Methods. sEV from maternal plasma of normal-pregnancies were isolated using tangential flow-filtration (TFF), size-exclusion (SEC) and PLAP-affinity chromatography based on its phosphatase activity. Their common features were characterized using immunoblot, nanotracking-analysis, and transmission-electron microscopy. The number/percentage EV-PLAP+ in the maternal plasma was determined using a modified flow cytometry (FC) protocol. The study protocol was approved by the Scientific Ethics Committee of the Pontificia Universidad Católica de Chile (#210618011). Results. We successfully obtained sEV-PLAP+ with high purity as demonstrated by the absence of classical lipoprotein marker ApoB and albumin, high yield and integrity. The percentage of sEV-PLAP+ in third-trimester MP was determined through FC. Conclusion. We established a reproducible method to obtain sEV-PLAP+ with high purity and yield from a pre-purified total sEV from MP can be obtained through a combination of SEC and TFF techniques. Additionally, the isolation and quantification of the percentage of EV-PLAP+ can be achieved using a modified FC protocol. These techniques can be utilized for early-stage diagnostic purposes in pregnancy-related pathologies.

Keywords: Placental small extracellular vesicles, placenta, pregnancy syndromes

Financing: Fondecyt Regular #1221362, Chile. CONICYT, PAI Convocatoria Nacional Subvención a Instalación en la Academia, Convocatoria Año 2019, Folio N°PAI77190060 to DC.

Metformin and female reproduction: effects on physiological and pathological conditions

Candela Velazquez[1], Yamila Herrero[1], Maria Silvia Bianchi[1], Mayra Bordaquievich[1], Melanie Neira[1], Débora Juana Cohen[1], Patricia Cuasnicu[1], Katherine Prost[2], Rocío Marinoni[1], Fernanda Parborell[1], Dalhia Abramovich[1]

(1) Instituto de Biología y Medicina Experimental- CONICET-, Vuelta de Obligado 2490, Buenos Aires, Argentina. (2) 2Hospital Interzonal General de Agudos Pedro Fiorito, Av. Manuel Belgrano 827, Avellaneda, Buenos Aires, Argentina.

Background: Metformin is a hypoglycemic drug used for type-2 diabetes that affects female reproductive system through unclear mechanisms. Since female fertility depends on energy balance and blood supply, a high-fat diet (HFD) could have detrimental consequences that metformin may improve. Methods: The Animal Experimentation Committee of the IByME-CONICET-approved the experiments done involving animals (Protocol Number 36/2017). 14-weeks old C57BL/6 mice were divided into two groups. One group received metformin orally. The other, water only. After 4 weeks, animals were split into 3 subsets. In one subset, blood and the ovaries were collected to measure hormones and follicular dynamic. The other subset was mated with males and the time to pregnancy and offspring features were analyzed. The third subset underwent in vitro fertilization (IVF). 21-days old C57BL/6 mice were fed for 15 weeks with regular chow or HFD. Body weight was determined once a week. At week 11, some HFD animals received metformin orally, for 4 weeks. Then, animals from the three groups were split and analyzed as explained in A. Results: Metformin reduced primary follicles, pup's weight, ovarian VEGF and progesterone levels. Estradiol levels and preantral follicles were higher. No differences were found after IVF. HFD mice had higher body weight, adipose tissue, ovarian VEGF, time to pregnancy and progesterone levels, with no differences in estradiol. We found a smaller number of pups per litter with no differences in pups' weight. Metformin restored these alterations. The % of newly-formed corpora lutea was lower in HFD. Metformin increased this % to control values. After IVF, fewer oocytes were retrieved, with no effect of metformin. Conclusions: Metformin has effects on ovarian performance in physiological and pathological conditions, being able to restore the majority of reproductive alterations caused by a HFD. Altered ovarian angiogenesis may be one of the mechanisms that explains HFD and metformin effects.

Financing: Préstamo BID PICT 2020 2111

Acknowledgments: Fundación Williams, Fundación Barón, Fundación IByME

Cav3.2 directly contributes to the refilling of the Endoplasmic Reticulum Ca2+ store at fertilization.

Sebastián Vergara[1], Isabel Vidal[2], Emily Lopes[3], Rafael A Fissore[3], Ingrid Carvacho[1]

(1) Universidad Católica del Maule, Medicina, Talca, Chile. (2) Universidad Católica del Maule, Centro de Investigación de Estudios Avanzados del Maule, Talca, Chile. (3) University of Massachusetts Amherst, Department of Veterinary and Animal Sciences, Amherst, United States of America

Background. Fertilization triggers a cascade of cellular processes known as “egg activation”, including pronucleus formation, resumption of meiosis, exocytosis of cortical granules, and initiation of Ca2+ oscillations. The Ca2+ oscillations are long-lasting periodic increases of intracellular Ca2+ that have influence in embryo developmental competence. During oocyte maturation, the intracellular Endoplasmic Reticulum (ER) Ca2+ stores are increased in preparation for fertilization. Cav3.2, a voltage gated Ca2+ channel has been shown to contribute to the ER Ca2+ accumulation during maturation. However, the role of Cav3.2 during fertilization has not been completely elucidate. Methods. The expression of the Cacna1h gene in GV oocytes and MII eggs was evaluated by RT-PCR. The Cav3.2 lalization in meiotic stages was evaluated by immunocytochemistry (n=12, n=9 and n=6; GV, MI and eggs respectively). The impact of Cav3.2 in ER-refilling after egg activation was assessed by the injection of D1ER Ca2+sensor (3) in WT (n=11) and TKO (n=12). Following in-vitro translation, the eggs were activated using PLCz mRNA and monitored via microfluorometry. Data were analyzed by Mann-Whitney t-test and considered significant when $p<0.05$. IACUC approved protocol 05/2022. Results. Cacna1h transcripts were found in GV oocytes and MII eggs. Inmunofluorescence analysis from immunocytochemistry reveal differences in the distribution of the protein in GV, MI and MII eggs, showing an accumulation of the channel in the center of the oocyte in GV, changing to a plasma membrane pattern in MI and MII. Microfluorometry analysis demonstrated that TKO eggs fail to refill Ca2+ at the same rate as WT eggs after the third intracellular Ca2+ rise. Conclusions. Our results show that Cav3.2 channel has a differential membrane expression during oocyte maturation. In addition, we show that Cav3.2 not only contributes to ER Ca2+ accumulation during oocyte maturation but also directly contributes to the ER refilling in response to fertilization.

Financing: FONDECYT regular 1221308. Acknowledgments: FONDEQUIP ANID EQM200122

Imaging analysis to characterize cortical granules in mouse eggs

Arnaldo Yáñez[1,2], Fernando Hinostroza[2,3,4], Alejandro Rojas[5], Ingrid Carvacho[2]

(1) Universidad Católica del Maule, Escuela de Ingeniería en Biotecnología, Facultad de Ciencias Agrarias y Forestales, Talca, Chile. (2) Universidad Católica del Maule, Departamento de Medicina Traslacional, Facultad de Medicina, Talca, Chile. (3) Universidad Católica del Maule, Centro de Investigaciones y Estudios Avanzados del Maule, Vicerrectoría de Investigación y Postgrado, Talca, Chile. (4) Universidad Católica del Maule, Centro de Investigación en Neuropsicología y Neurociencias Cognitivas, Facultad de Ciencias de la Salud, Talca, Chile. (5) Universidad Católica del Maule, Facultad de Ciencias Básicas, Talca, Chile

Background. Immature oocytes in germinal vesicle stage (GV stage), as well as oocytes ready to be fertilized, MII eggs, contain specialized vesicles called cortical granules. Cortical granules (CGs) are composed of proteases, glycoproteins and structural proteins. The number of CGs decreases with oocyte maturation and in response to fertilization, the CGs fuse with the plasma membrane and release their contents into the perivitelline space. In mammals, during the exocytosis of CGs, specific proteins are released, preventing the polyspermy. Polyspermy is the fusion of multiple sperm in the egg, and it is associated with lethal chromosome abnormalities and non-viable embryos. The mechanisms underlying CG exocytosis and blocking polyspermy, and how these are related to deficient number, distribution, or abnormal size of CGs have not been fully understood. Our work optimize the current methodology of CG quantification by comparing the CGs densities of egg cortex and cytoplasm, as well as their size and distribution, using confocal microscopy and image analyses. Methods. Six-to-sixteen-week-old females (C57BL/6) were superovulated with intraperitoneal (i.p.) injection of pregnant mare's serum gonadotropin followed by i.p. injection of human chorionic gonadotropin. After euthanasia, eggs were collected from the oviduct. Each egg was fixed and stained using LCA DyLight™649, mounted, and confocal 3D projection images were taken. Imaging analyses were done using Image J. IACUC approved protocol 05/2022. Results. A range of 4202-6809 CGs/egg was obtained, with a mean value of 5378 CG/egg, close to the value found in the literature (1,2). A higher frequency of size distribution from 0.30 to 0.45µm of CGs was observed. We found a highest concentration of CGs displaying a size of 0.35µm. Conclusions. The quantification, distribution and size of CGs will help us to assess the role of different proteins in the distribution, trafficking and exocytosis of CGs in preparation for, and in response to fertilization.

Keywords: mouse egg; quantification; cortical granules

Financing: FONDECYT Regular 1221308. Acknowledgments: FONDEQUIP-ANID EQM200122

Male Reproduction

Exogenous Rho GTPases modulation early and late affect human spermatozoa function and structure

Bárbara Rivera[1], Pamela Uribe[2], Alfonso Hernández[1], **Rodrigo Boguen[1]**

(1) Centro de Investigación en Salud de Precisión (CISP), Departamento de procesos Diagnósticos y Evaluación, Facultad de Ciencias de la Salud, Universidad Católica de Temuco, Temuco, Chile. (2) Departamento de Medicina Interna, Facultad de medicina, Universidad de La Frontera, Temuco, Chile

Background. Actin cytoskeleton is regulated by Rho GTPases in somatic cells (1). In spermatozoa, actin is found in head and surrounding the flagela (2). The effect of exogenous modulation of Rho GTPases in human spermatozoa has not been studied, so the aim of this work was to determine the early and late effect of Rho GTPase modulation on the function and structure of human spermatozoa. Methods. Selected spermatozoa (1x106/mL) were exposed to capacitating (CAP) or non-capacitating (NC) conditions. In each condition, spermatozoa were incubated at different concentrations of a Rho GTPase activator (ARho) and inhibitor (IRho) for 10 (T10) and 120 minutes (T120) at 37 °C. A direct contact group without incubation (T0) was added. Subsequently, motility, acrosome reaction (AR), plasma membrane lipid disorder and sperm morphology were evaluated. Data were analyzed by Two-way ANOVA and considered significant when $p<0.05$. This work has been approved by research ethics committee of Universidad Católica de Temuco (document 60/20). Results. Sperm progressive motility exposed to ARho and IRho in NC, decreased from T0, but in CAP this decrease was observed from T10, in parallel to an increase only on non-progressive motility. AR occurred from T0 with IRho only in CAP, but at T120 in all conditions the spermatozoa showed increased AR. Membrane lipid disorder in spermatozoa was only observed using IRho from T0 in CAP and NC. Abnormal sperm morphology increases in all experimental conditions, where tail and head defects occur frequently in NC and CAP respectively. In addition, the presence of pathological vacuoles only increases at T120 in both CAP and NC conditions. Conclusion. Exogenous modulation of Rho GTPases causes early and late effects in human sperm, however it depends if CAP or NOCAP condition are acting, as well as activator or inhibitor effect on Rho GTPase.

Keywords: Rho GTPase, Human spermatozoa, Sperm morphology

Funding. Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) N° 11201011, Agencia Nacional de Investigación y Desarrollo (ANID).

References1. Hodge RG, Ridley AJ. Nature reviews Molecular cell biology. 17(8):496-510. 2016.2. Mortimer D. Molecular human reproduction. 24(12):567-92. 2018

H_2O_2-induced mPTP opening in human spermatozoa causes mitochondrial dysfunction and cell death markers expression

Anita Bravo-Maldonado[1], Raúl Sánchez-Gutiérrez[1,2], Pamela Uribe-Catalán[1,3]

(1) Universidad de La Frontera, Center of Translational Medicine-Scientific and Technological Bioresource Nucleus (CEMT-BIOREN), Faculty of Medicine, Temuco, Chile. (2) Universidad de La Frontera, Department of Preclinical Science and (3) Department of Internal Medicine, Faculty of Medicine, Temuco, Chile

Background: The mitochondrial permeability transition pore (mPTP) is a multi-protein complex located in the mitochondria that regulates physiological and pathological cellular processes[1].In spermatozoa, mPTP opening is one of the main mechanisms involved in mitochondrial dysfunction, which negatively impacts energy supply and fertilizing capacity[2,3]. The mPTP opening has been associated in human spermatozoa with ionomycin-induced endogenous oxidative stress[4] and peroxynitrite-induced nitrosative stress[5], however, the effect of mPTP opening in sperm exposed to exogenous oxidative stress has not been evaluated. The aim of this study was evaluate the effect of H_2O_2-induced exogenous oxidative stress on mitochondrial function and markers of cell death expression. Methods: The semen samples were obtained from normozoospermic healthy donors (n=8) and selected by swim-up technique.They agreed to participate and signed an informed consent approved by the university's Scientific Ethics Committe. Selected human spermatozoa were incubated with 3 mM H_2O_2 for 60 minutes. Then, mitochondrial function and cell death markers were evaluated. Data of mitocondrial membrane potential (ΔΨm), ATP levels, mitochondrial ROS (mROS) levels and phosphatidylserine (PS) externalization, were analysed by a t-test. The one-way analysis of variance (ANOVA) was used to data analysed of mPTP opening and DNA fragmentation. The two-way ANOVA was used to intracellular Ca^{2+} concentration data analysed. A P value below 0.05 was considered statistically significant. Results: H_2O_2-induced exogenous oxidative stress caused sperm mitochondrial dysfunction, characterized by mPTP opening, increased intracelular Ca^{2+} concentration, decreased ATP levels, ΔΨm dissipation, and increased mitochondrial ROS production. Furthermore, H_2O_2-induced mPTP opening was associated with the expression of apoptotic cell death markers such as PS externalization and marginal DNA fragmentation. Conclusions: H_2O_2 exposure induces mitochondrial dysfunction mediated by mPTP opening leads to expression of apoptotic cell death markers The results of this study may explain the loss of sperm function in patients with infertility associated with oxitative/nitrosative stress.

Financing: ANID-FONDECYT: POSTDOCTORAL PROJECT GRANT 2023 Nº 3230085

Acknowledgments: The author acknowledge the financial support from ANID-FONDECYT: POSTDOCTORAL PROJECT GRANT 2023 Nº 3230085

Effect of incubation of thawed equine spermatozoa under different capacitating conditions on the sperm viability, membrane fluidity and intracellular Ca2

Paulina Cabrera Herreros[1,2], Luis Aguila[1], Macarena Castro[1], Ricardo Felmer[1,4], Maria Elena Arias[1,3]

(1) Universidad de La Frontera, Faculty of Agriculture and Environmental Sciences, Montevideo 0870, Temuco, Chile. (2) Universidad Santo Tomas, Facultad de Recursos Naturales y Medicina Veterinaria, Temuco, Chile. (3) Universidad de la Frontera, Department of Agricultural Production, Faculty of Agriculture and Environmental Sciences, Avenida Temuco, Chile. (4) Universidad de la Frontera, Department of Agricultural Sciences and Natural Resources, Faculty of Agriculture and Environmental Sciences, Temuco, Chile

Background. Ejaculated mammalian sperm acquire their ability to fertilize an oocyte during transit through the female reproductive tract, a process known as sperm capacitation. Induction of capacitation in vitro can be achieved by incubating spermatozoa with bicarbonate, calcium and albumin. However, in the equine species, in vitro sperm capacitation is not efficient1. The aim of this study was to evaluate the effect of incubating thawed equine spermatozoa in SP-TALP medium with different capacitation inducers. Methods. Spermatozoa were incubated for 120 min at 38.5°C in SP-TALP base medium supplemented with MβCD, IBMX, dbAMPc and the combination of IBMX and dbAMPc. SP-TLP without bicarbonate and albumin was used as a non-capacitating control. Acrosome reaction (PNA-FTC/PI), membrane fluidity (MC540) and intracellular Ca2+ (Fluo-3) were analyzed by flow cytometry. Semen was collected from three Chilote stallions 3–8 years old with a 'Colorado' artificial vagina twice a week during the reproductive season in South America (November 2018–January 2019) in compliance with the Universidad de La Frontera Scientific Ethics Committee (Act N° 057/2016). Results. Incubation of spermatozoa in capacitating conditions showed no differences in the acrosome reaction, however, greater membrane fluidity and intracellular Ca2+ was observed in capacitating treatments with capacitation inducers. Conclusions. The addition of capacitation inducers to the SP-TALP medium does not affect the integrity of the plasma membrane, achieving greater membrane fluidity, and intracellular Ca2+ levels, consistent with an increase in the capacitation of equine spermatozoa under these conditions. Future research will focus on analyzing the effect of these treatments on sperm capacitation parameters, such as tyrosine phosphorylation and zona pellucida binding capacity.

Keywords: Equine, spermatozoa, capacitation.

Financing: FONDECYT1160467, CONICYT, Chile.

Evaluation of molecular markers associated with ferroptosis in human spermatozoa subjected to oxidative stress

Pablo Contreras Mellado[1,2], Javiera Parada[1], Antonia Toro[1], Tiatira Torres[1], Claudia Moya[1], Osvaldo Merino[3,4], Jennie Risopatrón[3,4], Fabiola Zambrano[1,5], Raúl Sánchez[1,5], Pamela Uribe[1,6]

(1) Universidad de la Frontera (UFRO), Center of Excellence in Translational Medicine (CEMT-BIOREN), Faculty of Medicine, Temuco, Chile. (2) UFRO, Ph.D. Program in Sciences mention Applied Cell and Molecular Biology, Faculty of Agricultural Sciences and Environment, Temuco, Chile. (3) UFRO, Center of Excellence of Biotechnology in Reproduction (BIOREN-CEBIOR). (4) Department of Basic Sciences, (5) Department of Preclinical Sciences and (6) Department of Internal Medicine, Faculty of Medicine, UFRO, Temuco, Chile

Background. Ferroptosis is a reactive oxygen species (ROS)- dependent cell death characterized by GPX4 depletion, increased iron bioavailability, and oxidation of polyunsaturated fatty acids[1]. Owing to the unique architecture of the spermatozoon, it is exceptionally vulnerable to oxidative stress due to the presence of limited antioxidant defenses and a large number of oxidizable substrates[2]. Studies have shown that ferroptosis can be induced in germ cells, and their inhibition by pharmacological agents may be a potential strategy to treat or prevent male reproductive diseases associated with infertility[3]. However, ferroptosis-driven cell death mechanism in human spermatozoa exposed to oxidative stress in vitro has not been described. The objective of this study was to evaluate the effect of oxidative stress in ferroptotic cell death in human spermatozoa. Methods. The study was approved by the Scientific Ethics Committee (CEC-UFRO). Spermatozoa were exposed to arachidonic acid (AA) (5, 25, and 50 µM) for 1 h at 37ºC, including an untreated control. Parameters associated to oxidative stress were evaluated including ROS production (by DHE) and mitochondrial membrane potential (ΔΨm, by TMRM), as well as ferroptotic cell death markers including viability (by SYTOX Green), lipid peroxidation (by BODIPY C11), expression levels of SLC7A11, IREB2, GPX4 (by immunofluorescence), and iron content (by absorbance). All analyses were carried out by flow cytometry and microplate reader. Results. AA leads to oxidative stress evidenced by an increase in ROS production and impairment of ΔΨm; which was associated to cell death characterized by an increase in lipid peroxidation, IREB2, SLC7A11, and iron content, and a decrease in GPX4, compared to the untreated control. Conclusions. Oxidative stress induces cell death with biochemical characteristics of ferroptosis. This demonstrates another mechanism of impaired male fertility induced by oxidative stress and could establish new therapeutic targets to prevent the oxidative stress-mediated decrease in sperm quality.

Financing: FONDECYT 1230410, 11200955 & FONDECYT de iniciación 11201011

Environmental pollutant 3-methylcholanthrene may accentuate adverse reproductive effects of overnutrition in male rats.

Jeremias Pablo Flores Quiroga[1], Maria Agustina Meneghini[1], Florencia Heinecke[1], Marina Labiano[1], Rocío Alejandra Galarza[2], Veronica White[1], Alicia Graciela Faletti[1]

(1) CEFYBO-CONICET-UBA, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Ciudad Autónoma de Buenos Aires, Argentina. (2) CEREGeo-FCyT-UADER, Facultad de Ciencia y Tecnología, Universidad Autónoma de Entre Ríos, Ruta 11 km 10.5, Oro Verde, Entre Ríos, Argentina

The aim of this study was to investigate whether exposure to the environmental pollutant 3-methylcholanthrene (3MC), considered to be an obesogenic agent, can enhance the adverse effects of overnutrition on reproductive development on male rat. To this end, prepubertal male rats fed standard or cafeteria diets were exposed to vehicle, 3MC (0.1 mg/kg), alpha-naphthoflavone (αNF, 80 mg/kg) or a combination of them three times a week for 40 days. The study consisted of five experimental groups: controls (SDV), overweight rats (CDV), 3MC-exposed overweight rats (CD3MC), 3MC+αNF-exposed overweight rats (CD3MCαNF) and αNF-exposed overweight rats (CDαNF). At 61 days of age, blood, bone marrow and spermatozoa were collected. Sperm count (million spermatozoa/ml), motility (percentage), morphology by Giemsa staining, presence of DNA fragmentation in lymphocytes by comet assay and metabolic profile were assessed. CICUAL of the Faculty of Medicine-UBA approved by resolution nº 768/2021. CDV rats showed a higher body weight (387±7 g; $p<0.05$) than SDV rats (349±10 g), and 3MC and αNF reduced this increase to SDV levels ($p<0.05$). Cholesterol, glucose and triglycerides circulating levels showed no differences between the groups. Compared to SDV rats (37±1), all CD rats had lower sperm counts (CDV: 16±1; CD3MC: 13±2; CD3MCαNF: 15±3; CDαNF: 13±1; $p<0.001$). The CD groups also showed a decrease in motility percentage compared to SDV rats (15-50%). Furthermore, the CD3MC group showed a greater decrease in the number of motile spermatozoa (34±2; $p<0.001$) and higher tail DNA content (2.7±0.7; $p<0.05$) compared to CDV rats (53±3; 0.9±0.2; respectively). Interestingly, αNF prevented this effect (52±2; 0.26±0.01; respectively). All groups exposed to αNF (43.0±0.2 days; $p<0.05$) showed early puberty compared to SDV rats (44.2±0.2 days). These results indicate that overnutrition and 3MC can lead to reproductive anomalies. Moreover, overnutrition may increase the susceptibility of developing organisms to environmental pollutants and impair reproductive function.

Financing: Préstamo BID PICT 2018-01589, CONICET. Préstamo BID PICT 2020 serie A 01125, CONICET.

Temperature increase and response of heat shock proteins (HSP70, HSP90) in salmonid spermatozoa.

Osvaldo S. Merino Painen[1,2], Jennie M. Risopatrón González[1,2], Maxsihel A. Merino Merino[1], Claudio A. Vásquez Vásquez[4,5], Elías G. Figueroa Villalobos[3], Jorge G. Farías Avendaño[6]

(1) Center of Excellence of Biotechnology in Reproduction (BIOREN-CEBIOR), Faculty of Medicine, University of La Frontera (UFRO), Temuco, Chile. (2) UFRO Department of Basic Sciences, Faculty of Medicine, Temuco, Chile. (3) Catholic University of Temuco, Nucleus of Research in Food Production, Faculty of Natural Resources, Rudecindo Ortega, Temuco, Chile. (4) UFRO, Scientific and Technological Center on Bioresources (BIOREN), Temuco, Chile. (5) UFRO, Ciencias Agropecuarias y Medioambiente, Doctorate in Sciences, Temuco, Chile. (6) UFRO, Faculty of Engineering and Science, Temuco, Chile

Background: Climatic change increase in water temperature is causing intense, rapid and increasingly frequent changes in the aquaculture environment, has generated massive mortality of different species of fish both in the natural environment and captivity and has become one of the most severe problems in the aquaculture industry. Fish possess the Heat Shock Proteins (HSPs) to provide protection from harmful environments and their expression can rapidly increase in response to environmental stresses and thus protect proteins and cells from damage when under stressful conditions. However, the presence of HSPs in spermatozoa of Salmo salar and Oncorhynchus kisutch has not been described. Methods: we conducted a gene expression analysis of the HSP70 and HSP90 genes in Salmo salar and Oncorhynchus kisutch sperm. Fish spermatozoa were exposed at different in vitro temperatures (8–20°C) of the swimming medium and subsequently flash stored at -20°C to avoid variations in the expression of HSP genes. tRNA was extracted from sperm after temperature stress, and the modulation of HSP70 and HSP90 expression was assessed by RT-qPCR. The study was approved by CEC University of La Frontera (Protocol N°016/21; Act N°041_21). Results: Our study detected HSP70 and HSP90 mRNA in all activation temperatures tested at different levels in sperm in both species. It was significantly up-regulated by heat stress ($P<0.05$), except that no significant difference was found in temperatures at 16°C ($P>0.05$) in Salmo salar sperm. HSP70 and HSP90 expression were downregulated in Oncorhynchus kisutch sperm during the heat shock treatment. Conclusions: We identified HSP70 and HSP90, genes in Salmo salar and Oncorhynchus kisutch sperm. Furthermore, the expression profiling of data showed the expression pattern of HSP70 and HSP90 genes in both species of fish, under high-temperature stress, which may be associated with functionality under heat stress conditions.

Financing: Funding: ANID-FONDECYT POSTDOCTORADO 3210593 - FONDECYT INICIACIÓN 11230690. Acknowledgments: Hendrix Genetics S.A and Salmones Aysén S.A for providing biological material.

Progesterone induces the physiological opening of the mitochondrial permeability transition pore (mPTP) in human spermatozoa

Javiera Parada[1,2], Antonia Toro[1,2], Tiatira Torres[1,2], Pablo Contreras[2], Fabiola Zambrano[3], Pamela Uribe[3,4]

(1) Universidad de La Frontera, Medical Technology Career, Faculty of Medicine, Temuco, Chile. (2) Universidad de La Frontera, Center of Excellence in Translational Medicine, Scientific and Technological Bioresource Nucleus (CEMT – BIOREN), Temuco, Chile. (3) Universidad de La Frontera, Department of Preclinical Sciences, Faculty of Medicine, Temuco, Chile. (4) Universidad de La Frontera, Department of Internal Medicine, Faculty of Medicine, Temuco, Chile

Background. Mitochondria in eukaryotic cells participate in various cellular processes, including the regulation of cytosolic Ca^{2+} concentration. A pathway for Ca2+ release from mitochondria relies on the mitochondrial permeability transition pore (mPTP). The mPTP can open at two different configurations, one at high conductance with deleterious consequences to cell homeostasis and the second one at low conductance, also called physiological mPTP flickering, which allows Ca^{2+} efflux from the mitochondrial matrix, participating in Ca^{2+}-mediated signaling. In sperm cells, Ca^{2+} signaling is particularly important during the physiological progesterone response, and the mechanisms underlying this response are not fully understood. Currently, there are only a few reports related to the mPTP in spermatozoa, which are centered on the pathological role of mPTP opening; however, the physiological role of transient mPTP opening in human sperm has not been described. This project aims to characterize the mPTP opening in response to progesterone in human sperm. Methods. For this, motile human spermatozoa from normozoospermic donors were selected by swim up technique. The sperm mPTP opening induced by different concentrations of progesterone (0.001 – 2000 mM) was assessed through the calcein-cobalt method. Controls included untreated sperm, ionomycin (which induced mPTP opening), and vehicle. In addition, sperm quality parameters including viability, mitochondrial membrane potential (MMP), ROS production and phosphatidylserine (PS) externalization were evaluated. All parameters were analyzed by flow cytometry. The study was approved by the Ethics Committee of the University of La Frontera. Results. Progesterone induces the mPTP opening in human spermatozoa without affecting viability, MMP or ROS production, while PS externalization was slightly increased compared to the untreated control. Conclusions. Progesterone induces the mPTP opening in human spermatozoa and this process is physiological since sperm quality parameters are not affected. This suggests that the mPTP opening participates in the biological response to progesterone in human spermatozoa.

Financing: Grant to support for sponsoring professors of undergraduate and postgraduate students, 2023 call, Universidad de La Frontera.

Germ cell differentiation of adipose-derived bovine mesenchymal stem cells in testicular organoids

Jahaira Cortez[1,2], Cristian Torres[1], Víctor H Parraguez[1], Mónica De los Reyes[1], **Oscar A Peralta[1,3,4]**

(1) University of Chile, Faculty of Veterinary and Animal Sciences, Santiago, Chile. (2) Doctorate Program of Forestry, Agriculture, and Veterinary Sciences (DCSAV), University of Chile, Santiago, Chile. (3) Catholic University of Chile, School of Veterinary Medicine, Santiago, Chile. (4) Correspondence: oscar.peralta@uc.cl

Background. Germ cell (GC) in vitro derivation and transplantation have potential applications in bovine reproduction, as an alternative method for dissemination of elite animal genetics, production of transgenic animals, and conservation of endangered breeds. Our group has recently reported that bovine fetal mesenchymal stem cells (bfMSC) may be candidates for GC derivation and transplantation mainly based on their reduced immunogenicity and in vitro potential for transdifferentiation. Here, the in vitro GC differentiation potential of bfMSC was evaluated using a bovine testicular organoid (TO) generated from primary testicular cells. Methods. Bovine fetal MSC were obtained from fetal adipose tissue (AT-MSC). Leydig, Sertoli and peritubular myoid cells were isolated from adult bull testis. PKH26-positive MSC-TA and testicular cells were co-suspended at a concentration of 1x106 cells in DMEM-F12-based medium using Ultra-Low Attachment U-shape plates and cultured at 37°C in 5% CO2 for 28 days. TO were evaluated using confocal microscopy at days 0, 16, and 28 to assess the location of testicular cells (Star, WT1 and Col1a) and PKH26-MSC-TA. Results. TO containing MSC-TA (TO+MSC-TA) were smaller ($P<0.05$) (604 - 864 µm) compared to TO without AT-MSC (TO) (555.15 - 1272.88 µm). Star-positive (Leydig) cells were located in the peripheral area of TO and TO+MSC-TA, while MSC-TA were detected in the central region of TO+MSC-TA. In both TO and TO+MSC-AT, WT1-positive (Sertoli) cells were detected in the central area; however, in TO+MSC-AT, WT1-positive cells were segregated by MSC-TA. Col1a-positive (peritubular myoid) cells decreased in the central area of TO during culture; however, they remained in TO+MSC-TA until day 28 of culture. Conclusions. AT-MSC and testicular cells were able to self-assemble into a TO. AT-MSC adopted a central location modifying the structure of the TO inducing a peripheral location of Leydig cells and a central location of Sertoli cells and peritubular myoid cells.

Keywords: Germ cell differentiation, Mesenchymal stem cell, Testicular organoid

Financing: Fondecyt 1191114 & 1231145

Liraglutide regulates Sertoli cell energetic metabolism

Marina E Dasso[1], Cecilia L Centola[1], María Noel Galardo[1], Silvina B Meroni[1], **Maria Fernanda Riera[1]**

(1) Centro de Investigaciones Endocrinológicas "Dr César Bergadá" (CEDIE-CONICET), Hospital de Niños Ricardo Gutiérrez, Gallo 1330, Ciudad de BS AS, Argentina

Background: Sertoli cells (SC) provide the nutritional support for developing germ cells. They metabolize glucose to lactate, which is delivered to germ cells to be used as energy substrate. Besides, SC can use fatty acids (FA) as source of energy and can store them as triacylglycerols (TAG) within lipid droplets (LD). The LD content is important to maintain energetic reserve in SC. Obesity prevalence has risen dramatically and liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, has emerged as a useful drug for treatment of obesity. It has been observed that liraglutide regulates cell metabolism in different cell types. Considering that SC energetic metabolism may be relevant to seminiferous tubule physiology, the aim of this work was to assess if liraglutide regulates lactate production and LD storage in SC. Methods: Twenty-day-old rat SC cultures were incubated with 100 nM liraglutide for various periods of time. The protocol was approved by the Local Institutional Ethical Committee (CICUAL-Res#2018-01). Results: We showed that liraglutide does not modify glucose consumption, glucose transporter 1 expression or lactate production. However, liraglutide increases TAG levels and LD content ($p<0.05$). These effects are accompanied by an increase in mRNA levels of the fatty acid transporter CD36 (FAT/CD36), glycerol-3-phosphate-acyltransferases 3 (GPAT3) and perilipin 1 (PLIN1) ($p<0.05$). Considering that liraglutide acts through GLP-1 receptor, we evaluated the participation of cAMP/PKA signaling pathway. We show that a PKA inhibitor significantly decreases the upregulation LD content elicited by liraglutide. In addition, dbcAMP significantly increases LD number and FAT/CD36, GPAT3 and PLIN1 mRNA levels ($p<0.05$). Conclusion: In summary, liraglutide promotes lipid storage in SC by regulating the expression of genes involved in FA transport, TAG synthesis and LD formation. Having in mind the importance of lipid metabolism to fulfill SC energetic requirements we postulate that liraglutide might improve the energetic status of the seminiferous tubule.

Keywords: Testis, Sertoli cells, liraglutide, lipid droplets

Financing: PICT 2020-1061, PIP 2020-0162, PICT 2020-0642.

Rho GTPase activity determination during in vitro sperm capacitation.

Bárbara Rivera[1], Rodrigo Boguen[1]

(1) Centro de investigación en salud de precisión (CISP), Departamento de procesos Diagnósticos y Evaluación, Facultad de ciencias de la Salud, Universidad Católica de Temuco, Temuco, Chile

Background. RhoA, Rac1 and Cdc42 are the main members of the Rho GTPase superfamily (1). In somatic cells, these proteins are regulators of the actin cytoskeleton (2). Actin is important during sperm capacitation (3). Direct relationship between these GTPases and some mammal sperm capacitation has been studied, however, this has not been studied in human spermatozoa, therefore, the aim of this work was to determine the activity of RhoA, Rac1 and Cdc42 during human sperm capacitation in vitro. Methods. Selected spermatozoa (1x106/mL) were exposed to a capacitating and non-capacitating condition for 2 hours at 37°C. In both conditions, 1 µg/ml of activator (aRho) and 2 µg/ml of inhibitor (iRho) of Rho GTPase were added. Then, sperm proteins were extracted and RhoA, Rac1 and Cdc42 activities were measured by immunoassay. Additionally, Rho GTPase activity was measure at 10 minutes in both conditions to determine the early activity. This work has been approved by research ethics committee of Universidad Católica de Temuco (document 60/20). Results. At 2 hours, sperm RhoA activity was increased significantly with aRho in both experimental conditions, but in presence of iRho, the activity was maintained. In the capacitating condition sperm Rac1 activity was increased significantly with aRho and iRho, while in the non-capacitating condition this only occurred in the presence of aRho. Sperm Cdc42 activity was only increased with aRho in the non-capacitating condition. When measuring sperm RhoA and Rac1 activity at 10 minutes, they were similar in both experimental conditions, whereas in sperm Cdc42 activity was higher in the non-capacitating condition. Conclusion. Among the Rho GTPases studied, RhoA and Rac1 have early activity and during human sperm capacitation, and increase even more their activity in the presence of a Rho GTPase activator. Instead, Cdc42 showed activity only in the early stages of sperm capacitation and then decreased.

Keywords: Sperm capacitation, Rho GTPase, Human spermatozoa

Financing: Funding. Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) N° 11201011, Agencia Nacional de Investigación y Desarrollo (ANID).

Acknowledgments: References1.Feltri ML, et al. Glia. 56(14):1508-17. 20082.Hodge RG, Ridley AJ. Nature reviews Molecular cell biology. 17(8):496-510. 20163.Fleming AD, Yanagimachi R. The Journal of experimental zoology. 220(1):109-15. 1982

Regulation of intracellular Ca2+ in bovine spermatozoa: relevance of NCX and SERCA

Maia Rodriguez[1,3], Enrique L. Portiansky[1], Alejandro Orlowski[2], Paola Ferrero[2,3]

(1) UNLP, Facultad de Ciencias Veterinarias, La Plata, Argentina. (2) CONICET-UNLP, Centro de Investigaciones Cardiovasculares “Dr. Horacio E. Cingolani", La Plata, Argentina. (3) Universidad Nacional del Noroeste de la Provincia de Buenos Aires1, La Plata, Argentina

Background. Spermatozoa undergo biochemical and physiological changes known as capacitation, allowing them the ability to fertilize the oocyte. Modulation of the intracellular calcium (Ca^{2+i}) is essential for capacitation. This work studied the participation of the Na+/Ca^{2+} exchanger (NCX) and the Ca^{2+} ATPase pump (SERCA) in managing Ca^{2+i} in cryopreserved bovine spermatozoa. Methods. Cryopreserved bovine sperm (obtained through CIAVT) were thawed and resuspended in TALP medium. Subcellular localization of these proteins was evaluated in three different samples by indirect immunofluorescence, using anti-NCX and anti-SERCA primary antibodies and a cyanine-conjugated secondary antibody. Furthermore, we utilized inhibitors of NCX (KB-R7943) and SERCA (thapsigargin: Tg) to test their contribution to the regulation of Ca^{2+i} levels. We used the FLUO-3AM as Ca^{2+i} dye and a Varioskan multimode reader to detect the fluorescent signal. Fluorescence intensity was measured for 10 minutes in four groups: untreated or treated with Tg, KB-R, and Tg+KB-R. We calculated the fluorescence difference between the intensity recorded after 5 minutes and the initial intensity. The data were analyzed by one-way ANOVA. Results. The NCX was mainly detected in the post-acrosomal region, while the SERCA was observed in the acrosome. Treatment of spermatozoa with Tg and Tg + KB-R produced a significant increase in fluorescence intensity relative to control. However, we not detected changes in cells treated with KB-R alone. The combination of inhibitors slightly increased Ca^{2+i} compared to treatment with Tg alone. Conclusions. The results showed that SERCA, located in the acrosome, contributes significantly to the increase in cytosolic Ca^{2+i}. In contrast, the NCX, situated in the post-acrosomal region, plays a less relevant role, or its function might be compensated by other components not identified in this study promoting Ca^{2+} release out of cells while NCX is blocked.

Keywords: Bovine sperm, Calcium, SERCA, NCX

Financing: PIP2903 to Paola Ferrero

Crossover hotspots are characterized by a specific enrichment of histone modifications in the genome of mouse germ cells.

Katherine Urbina[1,2], Marcia Manterola[2]

(1) School of Medical Technology, Faculty of Medicine, University of Chile. (2) Human Genetics Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile.

Background. Meiotic crossovers (CO) are essential for reproduction and evolution. They ensure the proper chromosome segregation into gametes and promote genetic diversity. Defective CO formation leads to aneuploidy and infertility; hence, learning how CO are regulated is key to understand reproductive health and inheritance. In mammals, what defines the CO localization is still unknown. Here we explore if COs hotspots in mouse are patterned by a special epigenetic arrange composed by active chromatin marks such as H3K4me3 and H3K9ac. Methods. ChIP and Tiled qPCR were performed on spermatocytes from C57Bl6 mice. We analyzed 3 genomic regions: i) the CO hotspot of the X chromosome ii) a region of DNA DSBs formation but no CO production, iii) a region with no DSBs nor COs formation. Data analyses were performed using DDCt and statistical analyses were performed by t-student and ANOVA. Results. We found that specific histone modifications array shapes the different genomic regions analyzed. We found different H3K9ac and H3K4me3 occupancy in the DNA DSB, no-CO nor DSB and CO regions as well as the enrichment of nucleosomes that exhibited these histone marks. H3K4me3 was present as few peaks in the DSB, high peaks in the no CO region and in very low levels in the CO region. Contrarily, this region was fully occupied in nucleosomes with H3K9ac in low enrichment levels as compared with the other genomic regions. Thus, a particular landscape of active histone modification marks in the CO region that is different than other genomic sites. Conclusions. Our data show a specific epigenetic landscape in CO regions in the mouse genome. Moreover, suggest potential mechanisms by which histones regulates chromatin configuration and modulate the homeostasis of crossing overs, which are crucial for the correct achievement of gametogenesis and for the maintenance of genome stability in germ cells.

Keywords: Meiosis, Crossover, Epigenetic modifications

Financing: Fondecyt 11181329

Others

Types of delivery modify oxytocin receptor expression and signaling in the human placenta

Javiera Appel-Rubio[1], Vania Hormazabal Quilaman[2], Marco Loyola Brambilla[3], Yolanda Contreras García[2,4], Enrique Guzmán-Gutiérrez[1]

(1) Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Concepción, Chile. (2) Servicio de Obstetricia y Ginecología del Hospital Gran Benavente de Concepción, Concepción, Chile. (3) Laboratorio de Patología Veterinaria, Hospital Clínico Veterinario, Universidad de San Sebastián, Concepción, Chile. (4) Departamento de Obstetricia Y Puericultura, Facultad de Medicina, Universidad de Concepción, Concepción, Chile.

Background: Oxytocin is a fundamental hormone during pregnancy and childbirth, whose functions have been mainly described in excitable tissue such as the myometrium. There is evidence of an increase in its secretion and expression of its membrane receptor in the placenta as gestation progresses. There are also differences in hormone levels according to the type of delivery. However, their function and signaling mechanisms in placental tissue are still unknown. The general objective of this research was to characterize the oxytocinergic system in human placental explants according to the type of delivery. Method: From 20 placental samples classified according to type of delivery (n=5), human placental explants were prepared and administered oxytocin at increasing doses (0 to 10-9 M). OXTR expression was assessed by immunohistochemistry and eEF2 effector expression by western blot. Data were analyzed by Mann-Whitney, t-test or ANOVA, considering significance values $p<0.05$. This work had the approval of the bioethics committee and the informed consent of the patients. Results: In spontaneous labor, the OXTR expression decreases as oxytocin concentration increases until reaching 1nM, after which OXTR expression remains elevated. In contrast, in accelerated type deliveries, OXTR expression remains elevated at increasing doses of oxytocin. OXTR expression in cesarean section shows a tendency to decrease as oxytocin concentration increases. In spontaneous labor eEF2 is completely inactivated in contrast to the other types of labor. Conclusions: Our results suggest that tissue from spontaneous delivery would be sensitive to oxytocin changes within physiological levels, whereas increasing oxytocin to supraphysiological levels desensitizes OXTR and inactivates the signaling pathway, increasing eEF2 phosphorylation. In contrast, in explants from accelerated labor and cesarean section, doesn't change OXTR expression, but maintains the active signaling pathway. This is the first investigation that evaluates OXTR and it's signaling in human placentas, differentiating between types of delivery and the effect against oxytocin exposure.

Financing: Funding: VRID 220.84A.021-INV

Characterization of the adaptative immune response in HIV exposed uninfected (HEU) infants

Rodrigo Tonalli Camacho-Pacheco[1,2], Yesenia Brito-Pérez[1,2], Jessica Hernández-Pineda[1], Rocio Valeriano-Baena[1], Mextli Yoali Bermejo-Haro[1,2], Miguel Angel Nájera-Hernández[1,2], Jonatan Alejandro Mendoza-Ortega[1,2], Noemi Plazola-Camacho[1], Ricardo Figueroa-Damian[1], Diana Soriano-Becerril[1], Ismael Mancilla-Herrera[1]

(1) National Perinatology Institute, Immunology and Infectology, C. Montes Urales 800, Lomas, Mexico City, Mexico. (2) National Polytechnic Institute, National School of Biologic Science, Mexico city, Mexico

In utero viral exposure causes significant alterations on neonates development. HIV-exposed uninfected (HEU) newborns have higher rates of infections, morbidity, and mortality. Additionally, limited response to vaccination has been reported, as T helper (Th) cells proliferate and produce cytokines less after restimulation. Interestingly, HEU show inflammatory basal cytokine profile, reduced thymic size with less production of total T cells, and reduced specific antibody concentrations. We suggest that HEU infants born with an altered immune system, which would explain its increased infections susceptibility. Thereby, we evaluated, in HEU newborns, the total immunoglobulins concentrations, the frequency and phenotype of B, Th and dendritic cells, their functionality markers, and cytokine production. Also, Th cells, were stimulated, and differentiated to Th1 profile and reevaluated. This study was approved by the Research, Biosecurity and Ethics Committees at the National Institute of Perinatology, Mexico City, according to the ethical standards stated by the Declaration of Helsinki. Our results showed important alterations in IgG antibodies concentration in HEU newborns which are sustained along the first life year, showing higher IgG1 and IgG3 concentrations and lower IgG2. Regarding B cells, minor differences in the proportions of B lymphocyte subpopulations, but less phenotypic differentiation and lower proliferative capacity was found. About Th cells, we observed HEU infants born with less proportions of Th1, Th2, Th17 and Th1/17 cells, also they had less capacity to secrete IL-2 and IL-4 under polyclonal stimulation. Interestingly, under Th1 differentiation conditions they produced higher amounts of INF-γ. Even though dendritic cells, myeloid and plasmacytoid, were found in similar proportions to control groups, they possess activated phenotypes, expressing higher levels of CD80 and CD86; this correlated inversely with the Th cells proportions. In conclusion, our results suggested that *in utero* exposure to HIV generates a fetal programming that affects the development of its immune defense.

Keywords: HIV-exposed, Immune development, Adaptative immunology, Maternal programming

Financing: INPer: INPER-DG-0862-2021, INPER-2019-1-31; CONACyT scholarships: 1008800, 775561.

Development of a placental pathogenicity index for comprehensive assessment of maternal-fetal health and perinatal complications

Caroline Camilo[1], Gisele Rodrigues Gouveia[1], Veronica Luiza Vale Euclydes[1], Alexandra Brentani[2], Aloísio Souza Felipe da Silva[3,4], Helena Brentani[1]

(1) Faculdade de Medicina FMUSP, Departamento & Instituto de Psiquiatria, R., Sao Paulo, Brazil. (2) Faculdade de Medicina FMUSP, Departamento de Pediatria, Sao Paulo, Brazil. (3) Hospital Universitário da Universidade de São Paulo, Serviço de Anatomia Patológica, Sao Paulo, Brazil. (4) Faculdade de Medicina FMUSP, Sao Paulo, Brazil

Background. Placental pathologies are associated with maternal clinical alterations during pregnancy and adverse perinatal outcomes 1,2. Most studies analyze these pathologies individually or combining some groups of placental lesions, but there is no consensus on how to comprehensively assess placental health. Our proposal is the development of an index to estimate the pathogenicity of the different placental pathologies evaluated together. Methods. A subgroup of 481 births, with indication of placenta anatomopathological evaluation, from the São Paulo Western Region Birth Cohort (ROC) 3 were included in the study Placental lesions were divided in seven groups: Maternal Vascular Malperfusion (MVM), Fetal Vascular Malperfusion (FVM), Acute Inflammation (AI), Chronic Inflammation (CI), Villous Immaturity (VI), Chorangiosis (CHG) and Umbilical Cord alterations (UB) 4. Placental pathogenicity index has been developed with Structural Equation Modeling (SEM), using lavaan R-package. To test the index applicability, we select the Body mass index (BMI)/age at birth to show the relation between index and neonatal outcome. The study was approved by the HCFMUSP Research Ethics Committee (Process number: 5.450.815). Results. Placental pathogenicity index final model adequately fit between the hypothesized model and the sample data (Model fit= $\chi 2(12)$: 4.891, $p>0.05$, CFI: 1, TLI: 1, RMSEA: 0, SRMR: 0.02), considering all seven placental lesions groups. The model including BMI/age at birth as outcome also fit the models properly (Model fit= $\chi 2(18)$: 9.355, $p>0.05$, CFI: 1, TLI: 1, RMSEA: 0, SRMR: 0.02), showing a standardized coefficient of 0.14. Using linear regression models to evaluate if each placental lesion separately could be associated with BMI/age, we only found association for VI ($p<0.043$, adj. R 2 =0.01). Conclusions. An index that encompasses the multiplicity of placental lesions can improve understanding of maternal and fetal clinical alterations during intrauterine development, as well as provide better understanding of the physiopathology of perinatal complications.

Keywords: Placental pathology, structural equation modeling

Financing: Funding was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grants #2018/18560-6 and #2021/00607-9) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #310823/2021-8).

Evaluation of the immune response to the trivalent influenza vaccine in offspring of obese mice.

Mayra Castillo Ortega[1], Tomas Villaseñor Toledo[3], Fernando R. Esquivel Guadarrama[2], Gustavo Pedraza Alva[3], Delia Vanessa López Guerrero[1]

(1) Universidad Autónoma del Estado de Morelos, Laboratory of Immunology, Faculty of Nutrition, Morelos, México. (2) Universidad Autónoma del Estado de Morelos, Laboratory of Viral Immunology, Faculty of Medicine, Cuernavaca, México. (3) Universidad Nacional Autónoma de México, Institute of Biotechnology, Cuernavaca, Morelos, México

Background: Obesity is a global public health problem. It is associated with the development of cardiovascular diseases, type 2 diabetes mellitus, dyslipidemia, atherosclerosis and hypertension. Recently, obesity has been recognized as a risk factor for the development of complications during viral infections, such as influenza. The increasing prevalence of obesity in reproductive-age adults implies that parents may not be in optimal metabolic conditions at the time of procreation. As a result, offspring will be exposed to altered metabolic factors and a low-grade inflammatory environment, which predispose them to the development of metabolic diseases and immune system alteration (1,2). The aim of this study was to evaluate the efficacy of the trivalent influenza vaccine in offspring to obese mice challenged with the H3N2 influenza virus. Methods: C57BL/6J mice aged 6 to 8 weeks were used and kept under standard conditions. During mating and pregnancy, the mice received a high-fat diet for 8 weeks (D12492 from Research Diets). The offspring received a standard diet until weaning. Serum samples were collected on days 0 and 21 post-vaccination with the trivalent influenza vaccine. At 21 days, the mice were challenged with a lethal dose of the H3N2 influenza virus. All experimental procedures were reviewed and approved by the IBT-UNAM Bioethical Committee (project No.414). Results: The post-infection survival rate in offspring of parents on a control diet was 25%, while in the vaccinated group it was 50%. In offspring of obese mice, unvaccinated, the mortality rate was 100%, whereas in the vaccinated group it was 25%. The production of specific IgG antibodies against influenza post-vaccination was lower in offspring mice born to obese parents. Conclusions: The obesogenic environment in which the mice offspring develop decreases the efficacy of the trivalent influenza vaccine. The IgG anti-influenza is lower in offspring mice born to obese mice.

Keywords: Obese mice offspring, influenza vaccine, immune response

Financing: Funding: CONAHCYT, NATIONAL MASTER'S/2021 Scholarship No. 1145984

A classic randomized clinical trial to evaluate "mami-educ," an mHealth intervention for the primary prevention of gestational obesity: pilot results.

Jessica Moya[1], Fabian Pardo[2,3], Ayleen Bertini[4,5], Virla Palma[6], Andrea Rodríguez[6], Maricela Pino[7], Jaime Gutierrez[8], **Delia Indira Chiarello[8]**

(1) Universidad San Sebastian (USS), School of Nutrition and Dietetics, F. of Health Care Sciences, Santiago, Chile. (2) Universidad de Valparaiso (UV), Biomedical Research Center and (3) Faculty of Medicine, S. Felipe, Chile. (4) Millennium Institute for Intelligent Healthcare Engineering, Valparaíso, Chile. (5) UV, PhD en Ciencias e Ingeniería para la Salud, F. of Medicine, Valparaíso, Chile. (6) USS, School of Obstetrics, F. of Health Care Sciences, Santiago, Chile. (7) U. de las Américas, School of Obstetrics and Childcare, F. of Health and Social Sciences, Santiago, Chile. (8) USS, School of Medical Technology, F. of Medicine and Science, USS, Santiago, Chile.

Background. The promotion of healthy eating habits during pregnancy plays a crucial role in preventing the intergenerational transmission of obesity. In Chile, 69% of pregnant women are overweight or obese. Mobile health (mHealth) programs are potentially more effective than face-to-face interventions. Our aim was to develop a pilot mHealth intervention to reduce excessive weight gain in pregnant women attending primary care centers. Methods. A survey was administered before and after the intervention to evaluate the effectiveness in terms of knowledge about self-care and the perception of pregnant women regarding the use of digital tools. The intervention called mami-educ consisted of sending 3 weekly messages through the Multimedia Messaging Service, with dietary information for 12 weeks. A descriptive quantitative methodology was used in a group of 36 pregnant women between the 1st and 2nd trimester. The study was approved by the Scientific Ethics Committee of USS (protocol number: 07072020), Aconcagua Health Service (protocol #26/2021) and South Metropolitan Health Service (protocol # 07-27012022). Results. We observed a 52.2% reduction in excessive weight gain in obese pregnant women vs obese pregnant women in the control group. The intervention group increased nutritional knowledge by 15%, vs the group (routine care only). And 51% of pregnant women consider mobile messaging as an efficient means and prefer it over other means (digital or not) to receive health information. Conclusions. Mmessages have the potential to reduce excessive weight gain in obese pregnant women and can positively influence the nutritional self-care knowledge of pregnant women. This pilot intervention was extended in a classic randomized clinical trial (ClinicalTrials.gov Identifier: NCT05114174).

Financing: Institute of Public Policies of the San Sebastian University (IPSUSS1909). ANID-FONDEF, ANID- FONIS-7698SA21I0099. Acknowledgments: CESFAM Alberto Bachelet, Juanita Aguirre y Lucas Sierra, Canciller Orlando Letelier, Cóndores de Chile, Dr. Carlos Lorca, Santa Laura, Dr. Mario Salcedo, Dra. Haydée López Cassou, Segismundo Iturra.

Association between placental acute inflammation and telomere length at 12 months of life.

Gisele Gouveia[1], Caroline Camilo[1], Ketly Naiany Almeida Nascimento[1], Verônica Luiza Vale Euclydes[1], Aloísio Souza Felipe da Silva[2,3], Guilherme Vanoni Polanczyk[1], Helena Brentani[1]

(1) Faculdade de Medicina FMUSP, Departamento & Instituto de Psiquiatria, Universidade de São Paulo, São Paulo, Brasil. (2) Hospital Universitário da Universidade de São Paulo, Serviço de Anatomia Patológica, São Paulo, Brasil. (3) Universidade São Paulo, LIM/14, Faculdade de Medicina FMUSP, São Paulo, Brasil

Background. Longitudinal studies in animals and humans showed the importance of telomere biology in newborns and infants on long-term health. However, the determinants of telomere length (TL) in the first years of life are still not fully understood. Though heritability is high, known genetic variants represent a small proportion of observed variation, indicating that prenatal conditions may program TL1,2. Placental pathologies are related to worse outcomes in the offspring, but the link between these injuries and child TL has not yet been explored. Here we investigated the association between placental acute inflammation and TL at 12 months of age in children of a highly vulnerable group of mothers. Methods. A subsample of 36 individuals from two Randomized Clinical Trials (RCT) (NCT02807818 and NCT04362098)3 with high-risk pregnant adolescents enrolled in a psychosocial pre-parenting intervention in São Paulo, Brazil has placental tissue collected. All patients signed a consent form in accordance with ethical standards. Inflammatory lesions were classified by a pathologist considering maternal (acute chorionitis, acute amnionitis, and acute chorioamnionitis) and fetal (funisitis) response, according to Amsterdam4 criteria. Infant TL at 12 months of age was evaluated by the Real Time PCR5 as a potential biomarker of early life injury exposure. Linear regression models were performed using RStudio software, considering the RCT as a covariate. Results. Placental acute inflammation is associated with shorter telomeres at 12 months of life ($p<0.001$; adj. $R2=39$). This model remains, even after correction for variables that may be possible confounders, such as pre-gestational maternal BMI, child's gender, birth weight, and gestational age. Conclusions. Our study suggests a significant association between acute placental inflammation and telomere length in the first year of life. More factors that may be associated with placental health, such as chronic inflammation, maternal and fetal vascular malperfusion, need to be evaluated in future studies.

Keywords: Placental acute inflammation, telomere length.

Financing: Funding was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants 2018/18560-6 and #2021/00607-9) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 310823/2021-8).

Maternal and neonatal clinical variables and their association with necrotizing enterocolitis in preterm infants

Alma Leticia Hernández Martínez[1], Maria-Luisa Lazo-de-la-Vega-Monroy[1], Gloria Barbosa Sabanero[1], Claudine Liliane Irles Machuca[2], Ma. de la Cruz Ruiz Jaramillo[3], Adriana Dávalos Pérez[4], Guillermo Lozano Obregón[4]

(1) Medical Sciences Department, University of Guanajuato, México. (2) National Institute of Perinatology “Isidro Espinosa de los Reyes”, México. (3) León General Hospital, Guanajuato, México. (4) Health Sciences Division, University of Guanajuato, México

Objective: To assess maternal and preterm infant clinical data for the presence of necrotising enterocolitis. Methods: We observed newborns under 35 weeks gestational ages without chromosomopathies or malformations, their mothers don’t have preeclampsia, gestational diabetes, or infections at time of delivery, we collected their clinical information and the clinical information from their mothers. Arterial sampling of the umbilical cord is performed with a view to assessing plasma interleukin concentrations once the sample size is completed. Results: We observe sixteen newborns were classified as controls and three as cases of necrotizing enterocolitis. The cases of necrotizing enterocolitis were a newborns of 31.2±2.2 weeks of gestation, weighing 1445±335.07 g, the diagnosis of enterocolitis was made between the fifth and seventh day of life. In all cases are grade IIB enterocolitis accompanied by early sepsis. The 66.6% of the newborns in this group were exclusively breastfed and the 66.6% received mixed feeding. The sixteen preterm infants who are in the control group have an average gestational age of 34 ± 0.6 weeks, and weight of 2194.38 ± 242.43 g. The 75% of the newborns in this group were exclusively breastfed, the 6.25% received formula and 18.75% received mixed feeding. Leukocyte, neutrophil and platelet levels were assessed in both groups. Conclusions: Concentrations of the proteins of interest can’t be measured at this time because the sample size has not been completed, we compare maternal and neonatal clinical data in our group with necrotizing enterocolitis but we only have three participants, and the group must be larger to assess whether there is truly an association between clinical data and protein concentrations with the development of this disease.

Keywords: Necrotizing enterocolitis, premature birth, maternal clinical data, clinical neonatal data

PMN coincubated with sperm triggers neutrophil extracellular traps (NETs) and alters canine sperm functionality

Marion Leon[1], Claudia Moya[1], Rodrigo Rivera-Concha[1,2], Pamela Uribe[1,3], Aurora Prado[1], Antonia Toro[1], Raúl Sánchez[1,4], Carlos Hermosilla[5], Anja Taubert[5], Fabiola Zambrano[1]

(1) Universidad de La Frontera, Center of Excellence in Translational Medicine—Scientific and Technological Bioresource Nucleus (CEMT—BIOREN), Faculty of Medicine, Temuco, Chile. (2) Universidad de La Frontera, Ph.D. Program in Medical Sciences, Faculty of Medicine, Temuco, Chile. (3) Universidad de La Frontera, Department of Internal Medicine, Faculty of Medicine, Temuco, Chile. (4) Universidad de La Frontera, Department of Preclinical Sciences, Faculty of Medicine, Temuco, Chile. (5) Justus Liebig University Giessen, Institute of Parasitology, Giessen, Germany

Bakcground. Polymorphonuclear neutrophils (PMNs) are the first cells to reach sites of infection and inflammation. The mechanism of NETosis is currently known which releases extracellular traps (NETs) shaped by DNA, histones, and different antimicrobial proteins. The function of NETs is to capture and phagocytize microorganisms. Now, in reproduction it has been described that they act by trapping spermatozoa and causing damage to its functionality parameters, causing a negative effect on fertilization (1–3). However, there is no information on this mechanism in the reproduction of the canine species. Methods. All protocols were approved by the Scientific Ethics Committee of the Universidad de La Frontera with the authorization code 120_20 and were conducted in compliance with Chilean Animal Protection Statute No. 20.380. The canine PMNs isolated from peripheral blood were incubated with canine spermatozoa at a ratio of 1:3 for 15 minutes and 2 hours. Immunofluorescence was performed for NE, subsequent the visualization was trough under TissueFAXS I plus (TissueGnostics, Vienna, Austria) fluorescence microscope, and the analysis was using software StrataQuest 7.0. Proteins isolated from NETs were also coincubated with spermatozoa and the integrity of the spermatic membrane and the acrosome was evaluated using fluorescent probes SYBR14/PI y PNA-FITC/PI, respectively. Analysis was performed by flow cytometry. Results. The results allowed us to observe netotic PMNs in different phases, in addition to presenting different phenotypes of NETs trapping and neutralizing sperm. Also, the proteins of the NETs showed negative alterations against the parameters of the functionality of the sperm studied. Conclusions. This work demonstrates for the first time the presence of structures of NETs of PMN coincubated with sperm in the canine species. Also shows evidence of the cytotoxicity of proteins constituting NETs against sperm parameters in the species. This may be an important factor in the etiology of infertility, however, this idea requires further validation.

Keywords: Keywords. Neutrophil extracellular traps (NETs), sperm, canine.

Financing: ANID/CONICYT, FONDECYT Iniciación 11200955.

Increase of oral extracellular vesicles in pregnant women with gestational diabetes mellitus and periodontitis

María Luisa Mizgier[1,2], Ornella Realini[1,2], María José Bendek[1,2], Carolina Rojas[1,3], Sofia Monje[1], Aldo Figari[1], Marcela Hernandez[3,4], Valeria Ramirez[5], Dolores Busso[6,7], Sebastián Illanes[7], Alejandra Chaparro[1,2]

(1) Universidad de Los Andes (UAndes), Faculty of Dentistry, Santiago, Chile. (2) UAndes, Centre for Biomedical Research and Innovation (CIIB), Santiago, Chile. (3) University of Chile, Lab. of Periodontal Biology, Faculty of Dentistry, Santiago, Chile. (4) University of Chile, Department of Pathology and Oral Medicine, Faculty of Dentistry, Santiago, Chile. (5) UAndes, Department of Statistics and Epidemiology, Faculty of Dentistry, Santiago, Chile. (6) UAndes, Program in Biology of Reproduction, CIIB, Santiago, Chile. (7) IMPACT, Center of Interventional Medicine for Precision and Advanced Cellular Therapy, Santiago, Chile

Background: Recent studies suggest an association between periodontitis and gestational diabetes mellitus (GDM). However, the linked mechanisms involved in this association are poorly understood. We propose that extracellular vesicles (EVs) could be involved in the crosstalk between both diseases. Methods: 104 pregnant women were recruited at 24-32 gestational weeks. Clinical and periodontal data were recorded, and oral glucose tolerance test determined the GDM diagnose. Plasma and gingival crevicular fluid (GCF) samples were obtained. EVs were isolated by ExoquickTM or ultracentrifugation protocols, and then the morphology was characterized by Transmission electronic microscopy (TEM), concentration and size by nanoparticle tracking analysis, and EVs markers (CD81, CD9, CD63, syntenin-1, VL4-A, cytochrome c) were assessed by multiplex immunoassay. The ethical certification was approved by the Ethics Committee of the U. de los Andes. Results: From the 104 recruited women, n=18 of them (29±13 years, 30.14±13.19 BMI, kg/m2) were healthy (no periodontitis and no GDM), n=52 (29.5±12 years, 30.04±14.20 BMI, kg/m2) had periodontitis and n=34 (32±16.5 years, 31.43±9.32 BMI, kg/m2) had periodontitis and GDM. Total, small and large GCF-EVs concentration were increased in the group of pregnant women with periodontitis (p=0.0304) and periodontitis+GDM (p= 0.0015) compared to periodontal healthy pregnancies. However, these differences were not significantly in plasma-EVs between periodontitis+GDM vs periodontitis or healthy group. TEM shows that GCF and plasma EVs present a cup-shaped form as usually observed. EVs markers showed that GCF and plasma EVs expressed all the studied markers. CD9 and CD81 were decreased in GCF-EVs from pregnant women with periodontitis+GDM (p= 0.0462 and p= 0.0164, respectively) compared to healthy pregnancies. Conclusion: GDM and periodontitis induced an increased release of GCF-EVs, which might be involved in immune and inflammatory mechanisms which could be involved in intolerance glucose during pregnancy.

Financing: FONDECYT REGULAR 1211471

Neuroendocrine receptors involved in the regulation of the reproductive cycle of Seriola lalandi under captive conditions

Jaime Palomino[1], **Ayleen Olea[2],** Ricardo Moreno[3]

(1) Universidad Bernardo O'Higgins, Escuela de Medicina Veterinaria, Facultad de Ciencias Médicas, Avenida Viel 1497, Santiago, Chile. (2) Universidad de Chile, Laboratorio de Reproducción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Santa Rosa 11735, Santiago, Chile. (3) Pontificia Universidad Católica de Chile, Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Alameda 340, Santiago, Chile

Background: Seriola lalandi is the native fish with greatest prospect for Chilean aquaculture diversification. However, the mechanisms and molecules associated to reproductive cycle regulation and control in this species, which are fundamental to successfully management under captivity conditions, are still unknown. So, this work describes as first approach, the effect of environmental conditions on the expression of neuroendocrine receptors involved in the reproductive cycle of this species. Methods: Brain samples were obtained from adult individuals belonging to brood stock of Acuinor SA, Caldera. Samples were collected at different seasons for RNA extraction and cDNA obtaining. Primers of Melatonin, GnRH, Dopamine and Kisspeptin receptors genes (Melr, GnRHr, drd2 and Kissr, respectively) were designed and selected under standard procedures. Gene expression was assessed by RT-qPCR using relative expression. Differences in gene expression among seasons were analyzed by ANOVA and Tukey test ($p<0.05$). Results: For Melr, GnRH-1r and GnRH-2r the lowest expression level were detected in autumn, followed by a significant increase in winter, and a gradual decrease towards spring and summer. The expression of drd2 showed a similar pattern to receptors of hypothalamic and pituitary stimulant molecules, which was unexpected considering that dopamine has been described as inhibitory factor in several species. Finally, kiss1r displayed a similar pattern to stimulant molecules receptors, but kiss2r shows an expression stability from winter to summer, decreasing only at autumn. Conclusions: Low and high expression of Melr, GnRH-1r and GnRH-2r in autumn and winter seasons, respectively, would confirm the brain activation at short photoperiod condition after a reproductive rest. In addition, the results of the drd2 gene could indicate that dopamine doesn't have a regulatory control of reproductive cycle in this species. Finally, the different expression patterns of Kissr would indicate that Kisspeptins have a temporary role in the stimulation of the hypothalamic function in this species.

Financing: Funding: FONDECYT 1201343, 15PTEC-45861

Placenta, a promising source of bovine mesenchymal stem cells

Rudy Oyarzo Meynard[1,2], Catherine Jaramillo[1], Javiera Bahamonde Azcuy[1,3]

(1) Universidad Austral de Chile, Laboratorio de Morfología Aplicada, Facultad de Ciencias Veterinarias, Campus Isla Teja Universidad Austral de Chile, Valdivia, Chile. (2) Universidad Austral de Chile, Escuela de Graduados, Instituto de Farmacología y Morfofisiología, Campus Isla Teja Universidad Austral de Chile, Valdivia, Chile. (3) Virginia Tech, Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, 205 Duck Pond Drive VA-MD Vet Med (0442) Blacksburg, VA 24061, Blacksburg, United States

Background: Mesenchymal stem/stromal cells (MSC) have been studied mainly in companion or sport animals, thus little is known regarding capabilities of MSC of bovine origin, and even less if we refer to cells of fetal origin1. It has been possible to isolate fetal bovine MSC from different types of tissues, such as bone marrow (BM-MSC) and adipose tissue (AT-MSC), but the extraction protocols are highly invasive, and alternatives are needed. Tissues associated with parturition, such as cotyledons have been proposed as a viable source of MSC in bovines, without invasive protocols, risk to the mother or calf, or ethical concerns2. The aim of our study was to characterize bovine placental MSC (PT-MSC) and compare them against fetal bovine BM-MSC and AT-MSC. Methods: MSC were obtained from abattoir-derived fetuses (MO-MSC/AT-MSC n=7) and placentas (PT-MSC n=6)(no bioethics permit required), and cultured at 38,5°C with 5% CO2 in a humid atmosphere. Once cells reached 70-80% confluence in passage 2 (P2), they were detached, quantified using an automatic cell counter, and the viability, yield, size, time to reach P2, capacity of proliferation, migration and expression of certain bioactive factors were compared. Results: No significant differences in size were detected. Viability was statistically higher in AT-MSC compared to the other 2 tissues. However, PT-MSC exhibited significantly higher yield at P2 and took significantly less time to reach P2 compared to the other tissues, while BM-MSC had the lowest yield and took the longest to reach P2 compared to the other tissues. Proliferation, migration and expression of bioactive factors data is currently being analyzed. Conclusions: Cotyledons proved to be a reliable source of bovine MSC, with the ability to quickly reach high yields at P2. This could translate into higher ability to achieve therapeutic doses in less time, when translated to cell therapies.

Financing: National Agency for Research and Development (ANID, Project FONDECYT 11180681) and Escuela de Graduados, Facultad de Ciencias Veterinarias UACh.

Bioenergetic parameters in Treg lymphocytes from offspring gestated in maternal hypothyroxinemia induced with experimental autoimmune encephalomyelitis.

MA Rangel-Ramirez[1,2,3], E González-Madrid[1,2], A Matamoros[2,3], MJ Mendoza-León[1,2], O Álvarez-Mardones[1,2], E Caamaño[1,2], A Regaldiz[1,2], AA Elorza[2,3], CA Riedel[1,2]

(1) Laboratorio Endocrino-Inmunología, Departamento de Ciencias Biológicas, F. de Ciencias de la Vida, U. Andrés Bello, Santiago, Chile. (2) Instituto Milenio de Inmunología e Inmunoterapia (IMII), Santiago, Chile. (3) Laboratorio de Medicina Mitocondrial, Instituto de Ciencias Biomédicas, Fac. Medicina y Ciencias de la Vida, Santiago, Chile

Background: Maternal hypothyroxinemia (HTX), is a condition defined by low levels of thyroxine (T_4) and normal levels of T_3 and TSH. Regulatory T (T_{reg}) lymphocytes from offspring gestated in HTX (HTX-offspring) have reduced suppressive capacity both *in vitro* and challenged with Experimental Autoimmune Encephalomyelitis (EAE). Mitochondrial activity and metabolism oxidative are correlated with immunosuppressor function of T_{reg} lymphocytes. Bioenergetic parameters of T_{regs} from the HTX-offspring were analyzed to understand the reduced immunosuppressive function. Methods: HTX was induced in C57BL/6 pregnant mice. Adult offspring (HTX-offspring) was challenged with EAE at P55 and euthanized on day 10^{th} post-EAE induction, Spleen was recovered and $CD4^+CD25^+$ T lymphocytes (T_{reg}) were purified. Offspring gestated under euthyroidism (EUT-offspring) was Control group. Changes in the mitochondrial membrane potential (MMP) of T_{reg} were determined using the staining with TMRE. Flow cytometry analysis was performed on a BD FACSAria III. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured with Seahorse XFPro Analyzer. For that, T_{regs} were plated in XF V7-PS plates to measure basal respiration, Oligomycin was added to measure leaking, then FCCP was added to measure maximal respiration, followed rotenone/antimycinA were added to determine non-mitochondrial respiration. Glucose and 2-DG was added to measure glycolysis and glycolytic capacity. Statistical differences were tested using *one-way* ANOVA test. Results were shown as mean±SEM. This project was approved by the Universidad Andres Bello bioethics committee. Results: T_{reg} lymphocytes from HTX-offspring have altered MMP and altered bioenergetic parameters compared with T_{reg} lymphocytes from EUT-offspring. Altered basal and maximum respiration and high level of ECAR was observed in T_{reg} lymphocytes from HTX-offspring. Conclusions: T_{regs} from HTX-offspring have altered mitochondrial function and diminished oxidative metabolism mitochondrial, demonstrated by the measurement of bioenergetic parameters. This impairment could explain the decreased suppressive capacity of T_{reg} lymphocytes from HTX-offspring.

Financing: Beca UNAB. AAEC 2, 2023-2024, UNAB. ANID Doctorado #21202085; #21202356; #21211419. Acknowledgments: FONDECYT #1191300; #1180983. IMII ICN2021_045. Proyecto Núcleo UNAB DI-04-22/NUC.

Spermatozoa motility influences the generation of neutrophil extracellular traps – Preliminary results.

Rodrigo Rivera-Concha[1,2], Claudia Moya[1], Marion Leon[1], Antonia Toro[1], Iván Conejeros[3], Anja Taubert[3], Carlos Hermosilla[3], Raúl Sánchez[1,4], Fabiola Zambrano[1,4]

(1) Universidad de La Frontera, Centre of Excellence in Translational Medicine (CEMT), Faculty of Medicine, Av. Alemania 0548, Temuco, Chile. (2) Universidad de La Frontera, Ph. D. Program in Medical Sciences, Faculty of Medicine, Av. Francisco Salazar 01145, Temuco, Chile. (3) Justus-Liebig University of Giessen, Institute of Parasitology, Faculty of Veterinary Medicine, Schubertstrasse 81 35392, Giessen, Germany. (4) Universidad de La Frontera, Department of Preclinical Sciences, Faculty of Medicine, Manuel Montt 112, Temuco, Chile

Background: Infertility affects 15% of the world couples in reproductive age. infections/inflammatory diseases are a problem that causes a decrease in fertility. This decrease is linked to the presence of leukocytes in female reproductive tract (FRT), and polymorphonuclear neutrophils (PMN) are the first to arrive at infection/inflammation site. Human, swine, and bovine spermatozoa induces the release of neutrophil extracellular traps (NETs) in FRT and affects the sperm function. Our group have described that the sperm movement is one of the inductors for the release of NETs. It's known that a decrease in pH diminished sperm motility. The objective is to characterize the release of NETs associated to sperm motility. Methods: sperm samples were obtained from men by masturbation, and then selected by swim up with Sperm Preparation Medium to obtain motile sperm. Sperm cells were treated at pH= 4.0/5.0/6.0/7.4 by 30 min. PMN were isolated from human peripheral blood by Histopaque® 1119 density gradient. PMN were incubated with different sperm cells populations for 2 h. Extracellular DNA were detected by PicoGreenTM fluorescence and NETs were confirmed by fluorescence microscopy of Neutrophil Elastase and SYTOXTM Orange for DNA. Results: At pH=6.0 and in less extent pH=5.0 there's a trend to generate more anchored and cell-free extracellular DNA derived from PMN than control at pH=7.4. Sperm cells treated at pH=4.0 appears to activate PMN to release same quantity of extracellular DNA than at pH=7.4. Conclusions: Treatment of spermatozoa with pH=6.0 appears to generate more NETs, measure as extracellular DNA. The pH=4.0 treatment immobilize the sperm cells but generate same extracellular DNA than pH=7.4 treatment and that may be associated to a possible recovery in sperm motility after the treatment. Ethical Declarations: All protocols were approved by the Scientific Ethics Committee of the Universidad de La Frontera with authorisation code 120_20.

Financing: This work is funded by ANID/FONDECYT Iniciación 11200955 to Fabiola Zambrano. Rodrigo Rivera-Concha received an ANID/Doctorado Nacional/2020-21202442 scholarship. Acknowledgments: ANID FONDEQUIP EQM200228 for allowing us to use the TissueFAXS i Plus Cytometer microscopy facilities

Placental endocrine malfunction induces sex-specific changes in mitochondrial bioenergetics and related genes in mouse placenta.

Esteban Salazar-Petres[1,2], Jorge Lopez-Tello[1], Amanda Nancy Sferruzzi-Perri[1]

(1) University of Cambridge, Department of Physiology, Development and Neuroscience, Downing St Cambridge, CB2 3EG, Cambridge, United Kingdom. (2) Universidad Santo Tomás, Departamento de Ciencias Básicas, Facultad de Ciencias, Avda. Ramón Picarte 1160, Valdivia, Chile

Background. The placenta is an active endocrine organ that releases hormones with systemic effects on maternal physiology. However placental hormones may also act locally to modulate placental endocrine capacity, as well nutrient transfer function. Mitochondria are involved in placental steroidogenesis and energy production. The aim of this study was to assess the impact of induce placental endocrine malfunction on mitochondria function in the endocrine junctional zone (Jz) and transport labyrinth zone (Lz) of the mouse. Methods: Placental endocrine malfunction was achieved by selectively mis-expressing the imprinted Igf2 and H19 genes, which control placental endocrine cell formation and function; Jz-ICR1Δ (1–3). On pregnancy day 19, Jz and Lz tissues were dissected and cryo-preserved for assessment of mitochondrial function using high-resolution respirometry. Tissues were also snap-frozen for analysis of mitochondrial-related gene expression by quantitative PCR. Data were analysed for both fetal sexes and employed two-way ANOVA (genotype and sex as variables). Results. Compared to control, the Jz from female Jz-ICR1Δ placentas presented higher Complex ILeak, Complex I+IIOxphos and total electron transfer system respiratory rates. In contrast, no changes in respiratory capacity were detected in the Jz of male Jz-ICR1Δ placentas when compared to control. There were also no differences in Lz respiratory rates with Jz-ICR1Δ deletion in either sex. Despite this, Jz from male placentas showed decreased expression of Tfam, Nrf2, Opa1, Mfn1, Mfn2, Drp1 and Cyp1 due to Jz-ICR1Δ manipulation, while no differences were detected in females. Conversely, male Lz samples exhibited increased expression of Pparδ, Mfn2, Drp1, Fis1 and Cyp1, but only Fis1 was increased in female Lz in response to Jz-ICR1Δ. Conclusion. Placental endocrine dysfunction affects mitochondrial bioenergetics and related gene expression in different regions of the placenta in a manner dependent on fetal sex.

Financing: ES-P was supported by a Beca-Chile Postdoctoral-Fellowship. JL-T is supported by a Sir Henry Wellcome Postdoctoral-Fellowship. ANS-P is supported by a Lister Institute for Preventative Medicine Research Prize

Perinatal COVID-19 infection: relation with maternal infection and clinical features of neonates

Giovana Gonçalves[1], Letícia Dutra[2], Cibele Lebrão[3], Mariliza Silva[3], Fernando Fonseca[2], **Fabíola Suano-Souza[2]**

(1) Centro Universitário Faculdade de Medicina do ABC, Medical Student, Centro Universitário Faculdade de Medicina do ABC, Santo André, Brazil. (2) Centro Universitário Faculdade de Medicina do ABC, Department of Pediatrics, Centro Universitário Faculdade de Medicina do ABC, Santo André, Brazil. (3) Hospital Municipal Universitário de São Bernardo do Campo (HMU - SBC), Department of Pediatrics, Centro Universitário Faculdade de Medicina do ABC, Santo André, Brazil

Background. Pregnant women seem to have the mild or similar illness of COVID-19 symptoms as non-pregnant women. Prematurity is suggested to be one of the most common adverse effects of the Sars-CoV-2 infection during pregnancy. The consequences of COVID-19 infection during pregnancy and the repercussions in the short and long term for mother and child's health still need to be better understood. Methods. This study was approved by the Research Ethics Committee of Hospital Municipal de São Bernardo do Campo (HMU-SBC) and FMABC (CAAE: 34587520.3.0000.0082, opinion 4.184.253). A case-control study was carried out with 69 pairs of mothers who contracted COVID-19 during pregnancy and their neonates (23 cases who tested positive for Sars-CoV-2 in the first 48 hours of life and 46 controls who tested negative). Continuous variables were compared using the t-Student test and non-parametric ones were compared using the Mann-Whitney test. Categorical variables were compared using the chi-square test or Fisher's exact test. Results. The median of time between COVID-19 infection and delivery was 86 days (26.5; 179). There was no difference between the median of infection time and newborns RT-PCR positivity [positive vs negative: 92 (54,0; 233,0) days vs. 78,5 days (19,9;159,0); p = 0.086]. Most neonates were born by vaginal delivery at term and were appropriate for gestational age. Newborns who tested positive for RT-PCR were more likely to develop early respiratory distress (OR = 15.88; IC 95% 1.77 to 14.8) and oxygen use (p = 0.003) during hospitalization. Conclusion. The presence of early respiratory distress and the need for oxygen use were the most affected parameters due to COVID-19 perinatal infection. Vertical transmission, although apparently possible, is not certain, and further studies are necessary to confirm this hypothesis.

Financing: FAPESP – Regular Funding 2021/04784-2, FAPESP - research scholarship 2022/09207-6 and CAPES - research scholarship.

Acknowledgments: We are thankful for all the support given by São Paulo Research Foundation (FAPESP), Municipal Hospital of São Bernardo do Campo (HMU - SBC) and FMABC clinical analysis laboratory.

Maternal stress during pregnancy modifies circulating small extracellular vesicles and enhances their targeting to the fetoplacental unit

Mario Sánchez Rubio[1], Ana Del Valle[1], Lorena Abarzúa-Catalán[1], Maxs Méndez-Ruette[1,3], Lara Monteiro[2,3,5], Matias Pizarro[1], Soledad Sandoval[1], Roberto Romero[4], Ursula Wyneken[1,2,3], Sebastian Illanes[2,3,5], Federico Bátiz[1,2,3]

(1) Universidad de los Andes (UAndes), Neuroscience Program, Center for Biomedical Research and Innovation (CiiB), Santiago, Chile. (2) UAndes, Faculty of Medicine, Santiago, Chile. (3) UAndes, Center of Interventional Medicine for Precision and Advanced Cellular Therapy (IMPACT), Santiago, Chile. (4) NICHD/NIH, Perinatology Research Branch, Bethesda, USA. (5) UAndes, Biology of Reproduction Program, CiiB, Santiago, Chile

Background: Maternal psychological distress during pregnancy can negatively impact fetal development, resulting in long-lasting consequences for the offspring. These effects show a sex bias. The mechanisms whereby prenatal stress induces functional and/or structural changes in the placental-fetal unit remain poorly understood. Maternal circulating small extracellular vesicles (sEVs) are good candidates to act as "stress signals" in mother-to-fetus communication. Using a repetitive restraint-based rat model of prenatal stress, we examined circulating maternal sEVs under stress conditions and tested whether they could target placental-fetal tissues. Material and methods: Pregnant rats underwent 2hrs/day of movement restriction from E5.5 to E15.5 and were euthanized at E17.5. Plasma EVs concentration and size were quantified using NTA. Protein cargo of sEVs was Western blot analyzed. Maternal sEVs biodistribution in the fetoplacental unit was assessed by injecting DiR-labeled sEVs from stressed or control pregnant dams into females previously exposed to control or stress protocol. Procedures were approved by Universidad de los Andes Bioethical Committee and followed the NIH Guide for Care and Use of Lab Animals. Results: Our prenatal stress paradigm induced anhedonic-like behavior in pregnant dams and led to intrauterine growth restriction, particularly in male fetuses and placentas. Interestingly, specific stress-induced histological changes were observed in placental samples. The concentration and cargo of maternal circulating sEVs changed under stress conditions. Specifically, there was a significant reduction in neuron-enriched proteins and a significant increase in astrocyte-enriched proteins in blood-borne sEVs from stressed dams. Remarkably, maternal circulating sEVs target placental/fetal tissues and, under stress conditions, fetal tissues are more receptive to sEVs. Conclusion: Our results suggest that maternal circulating sEVs can act as novel mediators/modulators of mother-to-fetus stress communication. Further studies are needed to identify placental/fetal cellular targets of maternal sEVs and characterize their contribution to stress-induced sex-specific placental and fetal changes.

Financing: Fondecty-1211384(LFB)

Increase in child behavior problems among Uruguayan 1-4-year-olds: 2013 and 2018 ENDIS cohorts

Carlos Zunino[1], Danilo Dias Santana[3], Marianela Rodriguez Rey[2], Fernanda Blasina[2], Gustavo Giachetto Larraz[1], Alicia Matijasevich[3]

(1) Universidad de la República UDELAR, Clínica Pediátrica C. CHPR, Facultad de Medicina, Bvar. Artigas 1550, Montevideo, Uruguay. (2) Universidad de la República UDELAR, Unidad Académica Neonatología. Hospital de Clínicas., Facultad de Medicina, Av. Italia s/n, Montevideo, Uruguay. (3) Universidade de São Paulo, Departamento de Medicina Preventiva, São Paulo, Brasil

Background. Mental disorders in childhood are an important public health issue. Emotional and behavioral problems are present in children from an early age. A systematic review of studies from low and middle-income countries showed a prevalence of mental health problems in children and adolescents of about 10–20%, with anxiety, conduct, attention, and depressive disorders being the most common. These conditions often have their origins in childhood and adolescence, run throughout the life cycle, are associated with a wide range of adverse outcomes in adult life and carry substantial burden later in life. The aim of this study was to examine changes in behavioral and emotional problems among children aged 1-4 years, using data from the baseline of two Uruguayan cohort studies started in 2013 and 2018. Furthermore, we aimed to examine whether changes in emotional and behavioral problems could be accounted for by differences in socioeconomic, maternal and child characteristics between cohorts. Methods. We analyzed data from the baseline of the 2013 and 2018 ‘Encuesta de Nutrición, Desarrollo Infantil y Salud (Endis)’ cohort studies from Montevideo, Uruguay. A sample of 1-4-year-olds from the 2013 (n=790) and the 2018 (n=743) cohorts were assessed for behavioral/emotional problems through maternal reports using the Child Behavior Checklist (CBCL). Results. We found a significant increase in CBCL total problems, internalizing and externalizing mean scores over the 5-year period. Differences in maternal and child´s characteristics between the 2013 and 2018 cohorts were identified. After fully accounting for these differences, emotionally reactive, anxious/depressed, withdrawn and aggressive behavior scores remained evident in the analyses. Increases in the CBCL total and internalizing problems, anxiety/depression, withdrawal and sleep problems scores were largest among children from poor families. Conclusions. Our findings provide evidence for an increase in behavioral problems among young children in Uruguay over a 5-year period.

Keywords: Mental health, behavior problems, cohort study

Authors Index

V Meeting Latin American Regional Society for Developmental Origins of Health and Disease (DOHaD) and XXXIV Meeting Chilean Society of Reproduction and Development