
XXXV
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Universidad de Concepción

XXXV Reunión anual de la Sociedad Chilena de Reproducción y Desarrollo
XXXV Annual Meeting SChRD

Universidad de Concepción
25-28 September 2024

Auditorio Jaime Baeza de la Vicerrectoría de Investigación y Desarrollo de la Universidad de Concepción
Concepción, Chile

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Scientific program

Day 1 (Wednesday, September 25th)

Time	Activity	Speaker	Chair
15:00-15:15	Opening	SChRD President	Dolores Busso
15:15-16:00	**Conference 1. SCHRD 2023 Award "Artificial intelligence in obstetrics and gynecology: examples, opportunities and challenges"**	Daniela Mennickent- Universidad Católica de la Santísima Concepción, UCSC.	Héctor Contreras
16:00-17:30	**Symposium 1. "Impact of thyroid hormones during pregnancy and its offspring"** *"Gestational hypothyroxinemia triggers inflammation in maternal-fetal tissue"* *"Thyroid disorders in pregnancy and their effect on the resolution of infections in the offspring"* *"Placental Deiodinases in gestational diabetes: role of maternal Triiodethyronine"*	Claudia Riedel, U. Nacional Andrés Bello, Santiago, Chile Evelyn Jara, Universidad de Concepción, Chile Enrique Guzmán, Universidad de Concepción, Chile	Andrea Leiva
17:30-18:30	Coffee & Snacks		
18:30-19:30	**Conference 2. "Nutrient transport at the blood-brain barrier"**	Tom Arnold, University of California, San Francisco	Nicolás Santander

Day 2 (Thursday, September 26th)

Time	Activity	Speaker	Chair
9:00-10:30	**Symposium 2. "New therapeutic strategies and molecular targets for ovarian cancer"** *"Antitumor effects of microRNAs in different models of epithelial ovarian cancer"* *"Drug repurposing and ACSL4 inhibition as a possible complementary treatment for ovarian cancer"* *"Development of an alternative therapy against cisplatin-resistant ovarian cancer based on the use of retinoids and pro-oxidant agents"*	Carmen Romero, Universidad de Chile. Maritza Garrido, Universidad de Chile. Manuel Valenzuela Valderrama, Universidad Central de Chile.	Maritza Garrido
10:30-11:30	Coffee		
11:30-13:00	Short oral talks		Marcelo González
13.00-15.00	Lunch Break		
15:00-16:30	**Symposium 3. "Perinatal brain and systemic vascular function: the placental link with chronic adult diseases"** *"Maternal hypercholesterolemia: from placental function to future cardiovascular risk"* *"Effects of perinatal hypoxia on junctional molecule involved in the blood-brain barrier permeability"* *"Assessing metformin impact on maternal vasculature and pregnancy outcomes in a mouse model of a pregnancy complications"* *"Mechanosensitive mechanisms contributing to placental vascular function in health and disease"*	Andrea Leiva, Universidad San Sebastián, Santiago, Chile Alejandro González Candia, Universidad de O'Higgins, Rancagua, Chile Tamara Sáez Gutiérrez, Universidad de Valparaíso, Chile Bernardo J. Krause, Universidad de O'Higgins, Rancagua, Chile	Bernardo Krause
16.30-18.30	Posters + Coffee & Snacks		
18:30-19:30	**Conference 3. "Genomic tools to solve the mysteries of embryonic development"**	Ricardo Perecin Nociti Université de Montréal, Canadá	Luis Aguila

Day 3 (Friday, September 27th)

Time	Activity	Speaker	Chair
9:00-10:30	**Symposium 4. "Blood Brain Barrier development"** *"Placenta-brain communication in preeclampsia. role of extracellular vesicles in the disruption of the BBB"* *"The insect Blood-brain barrier as a model for understanding development and human diseases"* *"Cholesterol transport in the formation of the blood-brain barrier"*	Carlos Escudero. U. del Bío Bío, Chillán, Chile Esteban Contreras. Universidad de Concepción, Chile Nicolás Santander, Universidad de O´Higgins, Chile	Gareth Owen
10:30-11:30	Coffee		
11:30-13:00	Short oral and New Members talks		
13.00-15:00	Lunch Break		
15:00-16:00	**Conference 4. "Cortical granules and fertility: from basic biology to pathological conditions"**	Marcela Michaut U. Nacional de Cuyo, Argentina	Ricardo Fuentes
16:00-16:30	Coffee		
16:30-18:00	**Symposium 5. "Unconventional models in reproduction and development: expanding horizons using zebrafish, under-studied invertebrates and amphibians"** *"Studying germline evolution and polyspermy blockage beyond animal model systems: valuable insights from cnidarians, annelids, mollusks, and sea urchins"* *"Maternal genetics of the oocyte-to-embryo transition"* *"Zebrafish as a model to study the teratogenic potential of drugs and pollutants"* *"Participation of ATP- and Glutamate-mediated signaling in the formation of the neural tube in the chordate Xenopus laevis"*	Felipe Aguilera. Universidad de Concepción, Chile. Ricardo Fuentes. U. de Concepción, Chile. Javiera de la Paz Montt. Universidad de Concepción, Chile Patricio Castro, Universidad de Concepción, Chile	Bárbara Echiburú
18:00-19:00	**Closure Conference** *Creative Intersections: Art and Science in the Co-Creation Space"*	Marianela Camaño	Ingrid Carvacho
21:00-2:00	Awards Ceremony Farewell Dinner and Party	Hotel Araucano	

Day 4 (Saturday, September 28th): SChRD Members Meeting (hour and place TBC)

ABSTRACTS

CONFERENCES

***Conference 1*. SCHRD 2023 AWARD**

Artificial intelligence in obstetrics and gynecology: examples, opportunities and challenges.

Daniela Mennickent[1]
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Artificial intelligence (AI) is a scientific discipline that studies the theory and development of systems capable of simulating human intelligence, including its thinking capacity and behavior. This powerful tool has been applied to solve problems in various fields of knowledge, and healthcare is no exception. Indeed, some authors consider that AI will transform clinical practice in areas such as diagnosis, treatment, prognosis, and monitoring, among others.
This presentation will explore the utility of AI in obstetrics and gynecology, using the prediction of gestational diabetes mellitus and the study of the pathophysiology of endometriosis-associated infertility as concrete and illustrative examples of its potential. The presentation will emphasize the variety of data that can be utilized in healthcare applications, as well as the importance of interdisciplinarity and innovation in developing relevant AI-based solutions.
The presentation will showcase both original research results and literature findings that illustrate the potential of AI for studying gynecological and obstetric pathologies, and how this potential can extend to other human diseases. Additionally, the opportunities and challenges associated with implementing such strategies in clinical practice will be briefly discussed.
Keywords: Machine Learning, Pregnancy, Gestational Diabetes, Fertility, Endometriosis
Financing: Current financial support:Gobierno Regional del Bío-Bío: Project FIC-R BIP 40036152-0 “Capital Humano Avanzado en Inteligencia Artificial para el Bío-Bío”.Past financial support:Agencia Nacional de Investigación y Desarrollo: PhD Scholarship 21190736, together with projects FONDECYT 11170710, FONDECYT 11181153 and FOVI 210057.Universidad de Concepción and Ministerio de Educación: Project UCO 1866.
Acknowledgments: I thank my academic collaborators and the Reproductive Medicine Unit of Clínica Sanatorio Alemán.

Conference 2

Defective choline transport at the developing blood brain barrier causes congenital hydrocephalus

Thomas Arnold[1], Dibyanti Mukherjee[1], Rosemary Cater[2], Francesca Bertino[3], Nicolas Santander[4], Carlos Lizama[1], Filippo Mancia[2], Emanuela Tolosano[3], Deborah Chiabrando[3]
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(2) Columbia University, Department of Physiology and Cellular Biophysics, New York, USA
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Background: Hydrocephalus is a common disorder defined in its simplest form as the progressive accumulation of cerebral spinal fluid (CSF) within the cerebral ventricles, accompanied by ventricular dilation. Mutations in the Major Facilitator Superfamily (MFS) transporters FLVCR1 and FLVCR2 cause congenital hydrocephalus (CH), but the mechanisms mediating this relationship are unknown.
Methods: We employed cell culture and mouse models to evaluate the role of choline transporters Flvcr1 and Flvcr2 in the development of CH.
Results: Flvcr2 is expressed in the developing blood brain barrier (BBB) endothelial cells of mice and humans, while Flvcr1 is expressed primarily in brain neuroprogenitor cells (NPCs). We find that Flvcr1 and Flvcr2 transport choline into brain endothelial cells and NPCs, respectively. In vivo, conditional deletion of FLVCR1 from NPCs using NestinCre transgenic mice caused prenatal lethality and hydrocephalus, with defective NPC differentiation: reduced proliferation of radial glial cells (RGCs) and intermediate progenitors (IPs), and precocious neuronal differentiation. Conditional deletion of Flvcr2 from brain endothelium using Cdh5CreER resulted in similar defects in NPC proliferation/differentiation followed by progressive ventriculomegaly and pre- or peri-natal demise. Similar to humans with FLVCR2 mutation, Flvcr2 cKO mice displayed a distinctive glomeruloid vasculopathy. In contrast, blood vessels in FLVCR1 mutants were largely unaffected.
Conclusions: The phenotypic similarities between these mouse models and patients with FLVCR1/2 mutations, in tandem with their shared functional role as choline transporters, strongly suggest a unifying pathobiology centered around reduced blood-to-brain choline transport leading to NPC abnormalities and CH.
Keywords: Choline, Blood-brain barrier, Congenital hydrocephalus
Financing: NIH R01NS123168 A137796 and R21NS129105 A139793
Acknowledgments: Approved by IACUC at the University of California San Francisco

Conference 3

Genomic tools to solve the mysteries of embryonic development

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(2) Universidad de La Frontera, 2Laboratory of Reproduction, Centre of Reproductive Biotechnology (CEBIOR-BIOREN), Faculty of Agriculture and Environmental Sciences, Temuco, Chile

Gene regulation during embryonic development is a systematic process involving complex interactions between DNA, RNA, and proteins. Understanding these regulatory systems is essential, as they rule cell fate decisions, pluripotency, and orchestrate the formation of tissues and organs. Through genomics, we can probe how transcription factors, ncRNAs and epigenetic modifications engage with DNA to control the spatial and temporal expression of genes and proteins necessary for normal development. These can be done by comprehensive analysis of DNA (DNA-seq) and RNA (RNA-seq) sequences which reveal the genetic basis of cell metabolism. Moreover, Long-read sequencing can give insights into genomic regions, enabling detection of complex structural variants, full-length transcripts, and phased haplotypes. Also, ATAC-seq identifies open chromatin regions, highlighting regulatory elements for transcription factor binding. Furthermore, Methyl-seq enhances our understanding of the epigenetic regulation by mapping DNA methylation patterns, and ChIP-seq can map DNA regions bounded by specific proteins, such as transcription factors or histones, offering crucial insights into protein-DNA interactions. Additionally, direct RNA sequencing (dRNA-seq) preserves valuable physical base-specific information, including RNA length, modifications, poly(A) tail length, and alternative splicing. Moreover, Ribo-seq can capture mRNAs attached to ribosomes, thereby identifying proteins being synthesized. Likewise, Single-cell RNA sequencing (scRNA-seq) can provide high-resolution insights into gene expression at the individual cell level, identifying cell types and states. As well, Spatial Transcriptomics integrates gene expression with spatial data, mapping transcriptomic profiles to specific cell and tissue locations. All those technologies can be combined in a multiomic approach creating resources and interaction maps ranging from gametogenesis up to organ development. The future holds great promise by boosting our understanding of development, with enhanced methods and new tools to help researchers understand the mysteries of embryonic development in even more detail.
Keywords: Omics, embryo, development
Financing: FAPESP: 2019/04738-0, 2021/11912-7, 2021/09886-8; NSERC: CRDPJ536636-18, RGPIN-2020-05278; MITACS: IT15216; ANID-FONDECYT: 11230091 (LA)

Conference 4

Cortical granules and fertility: from basic biology to pathological conditions

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During mammalian fertilization, the fusion of cortical granules (CG) with the oocyte plasma membrane is one of the most significant events to prevent polyspermy and ensure embryo development. This secretion process, also known as the cortical reaction, is a highly calcium-regulated exocytosis and is thought to be mediated by the SNARE complex, involving key regulatory proteins such as α-SNAP and NSF. Our research has shown that α-SNAP and NSF are expressed in mouse oocytes, and that α-SNAP and NSF play active roles in CGE. Perturbations in these proteins, either through mutation or antibody microinjection, inhibit CG exocytosis (CGE), underscoring their importance in oocyte physiology. We also explored the role of R-SNAREs in CGE, identifying VAMP1 and VAMP3 as necessary components. These R-SNAREs, sensitive to tetanus toxin, are required for the fusion of cortical granules with the oocyte plasma membrane, further validating the involvement of the SNARE complex in this unique exocytosis. Further investigations into the role of Rab3A, a small GTP-binding protein, revealed that Rab3A co-localizes with cortical granules and is essential for their exocytosis. Microinjection of active Rab3A triggered CGE, while inhibition of Rab3A function abolished this process, highlighting Rab3A's critical role in cortical granule physiology. Additionally, our studies on α-SNAP mutations, particularly the M105I mutation found in hyh mice, have demonstrated its detrimental effects on oocyte quality and fertility. Hyh oocytes exhibit impaired CGE, abnormal cortical granule localization, and increased polyspermy rates, leading to reduced fertilization success. These findings emphasize the significance of α-SNAP in maintaining oocyte integrity and female fertility. In the context of assisted reproductive technologies, we examined the impact of in vitro aging on oocytes. Our results indicate that oxidative stress during in vitro culture leads to spontaneous CG release, reducing fertilization rates. However, supplementing the culture medium with dithiothreitol (DTT) prevented these negative effects by stabilizing the actin cytoskeleton and protecting key regulatory proteins of CGE, ultimately improving IVF outcomes. Our ongoing research, including unpublished data on oocyte quality in a mouse model of endometriosis, continues to expand our understanding of the molecular mechanisms underlying CGE and their implications for fertility.

SYMPOSIA

Symposium 1. **"Impact of thyroid hormones during pregnancy and its offspring"**

1. Searching for the mechanisms of gestational hypothyroxinemia that impair the neurodevelopment of the offspring

Enrique González[1], Sebastian Espinoza[3], Ignacio Cancino[3], Pablo Gonzalez[3], Alexis Kalergis[3], Susan Bueno[3], María Cecilia Opazo[2], **Claudia A Riedel**[1]
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Maternal thyroid hormones are essential for the proper development of the fetus, especially during the first 20 weeks of pregnancy. It has been shown that gestational hypothyroxinemia, a frequent maternal condition during early pregnancy characterized by low levels of thyroxine (T_4) and normal levels of triiodothyronine (T_3) and thyroid stimulating hormone (TSH), is strongly associated with cognitive damage like low IQ, attentional deficit, and autism spectrum disorder in the offspring. The mechanisms underlying the consequences of gestational hypothyroxinemia on the central nervous system (CNS) of the offspring are not precise yet. It has been proposed that low levels of T4 will provide less T3. Then, the reduction of T_3 will directly affect the gene expression of the differentiation cells from the CNS. Based on our experimental results, we proposed an alternative mechanism, where low levels of T_4 will induce maternal inflammation characterized by high levels of IL-6 that will damage the fetus's neurodevelopment and will prone the offspring to cognitive impairment.
Keywords: Gestational hypothyroxinemia, maternal inflammation, offspring, cognitive performance
Financing: Institute of Immunology and Immunotherapy, IMII ICN2021_045FONDECYT 1191300Núcleo UNAB, DI-04-22/NUC

2. Thyroid disorders in pregnancy and their effect on the resolution of infections in the offspring

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Background: Hypothyroxinemia (Hpx) is a pathology characterized by low Thyroxine (T4) levels without affecting Triiodothyronine (T3) and Thyroid Stimulating Hormone TSH. This becomes a problem during pregnancy because of the important requirement of T4 from the fetus for its development. It´s extensible proven that gestational Hpx has a negative effect in the central nervous system development and more recently in the immune system of the offspring. In this study, we evaluated whether adult mice gestated in HPX show an altered response to infection with the human Respiratory Syncytial Virus (hRSV).
Methods: BALB/cJ mice pregnant were treated with methimazole (MMI) in the drinking water during pregnancy day 10 (E10) to E15. The control group mice drank water without MMI. A third experimental group received MMI and T4 from E10 to E15. After of birth, mice of six-to 8-wk-old mice were anesthetized and challenged with hRSV. Body weight was recorded daily. Thyroid hormones of mice and their progeny were measured on the last day of treatment (E15) and at postnatal day 55 (P55) respectively. Seven days post infection (dpi), mice were terminally anesthetized for collection of bronchoalveolar lavages (BALs) and lungs for determination of viral load by qPCR, detection of cytokine secretion by ELISA, FACS analyses of BALs and lung, and lung histopathology.
Results: We observed that mice gestated under HPX condition showed a greater weight loss and increased viral loads in the lungs, as compared to mice gestated under euthyroid condition. In addition, the offspring gestated in HPX showed reduced secretion of interferon gamma (IFN-g), which correlated with fewer infiltrating CD8+ T cells in the lungs and BALs) without affect the infiltration of CD4+ T cells and neutrophils after hRSV infection. Finally, progeny gestated under HPX showed an increase in the lung inflammation after infection with hRSV similar to that observed in mice gestated in euthyroid condition and Hpx plus T4.
Conclusions: These data support the notion that a TH deficiency during gestation can "imprint" the offspring with an enhanced susceptibility to the pathology caused a respiratory virus.
Ethical declaration: Approved by the ethical committee of Pontifical Catholic University of Chile.
Keywords: Thyroid hormones, Pregnancy, Hypothyroxinemia
Financing: Fondecyt #3150559; FOVI230114 and ANID; 2021000369MUL VRID-UdeC

3. Placental Deiodinases in gestational diabetes: role of maternal triiodethyronine

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Gestational Diabetes Mellitus (GDM) is characterized by abnormal maternal D-glucose metabolism and altered insulin signaling. Dysregulation of thyroid hormones (TH) tri-iodethyronine (T_3) and L-thyroxine (T_4) Hormones had been associated with GDM, but the physiopathological meaning of these alterations is still unclear. Maternal TH cross the placenta through TH Transporters and their Deiodinases metabolize them to regulate fetal TH levels. Currently, the metabolism of TH in placentas with GDM is partially unknown. Therefore, we evaluated the levels of maternal TH during pregnancy, and fetal TH at delivery, and the expression and activity of placental deiodinases from GDM pregnancies. Pregnant women were followed through pregnancy until delivery. We collected blood samples during 10-14, 24-28, and 36-40 weeks of gestation for measure Thyroid-stimulating hormone (TSH), Free T_4 (FT_4), Total T_4 (TT_4), and Total T_3 (TT_3) concentrations from Normal Glucose Tolerance (NGT) and GDM mothers. Moreover, we measure fetal TSH, FT_4, TT_4, and TT_3 in total blood cord at the delivery. Also, we measured the placental expression of Deiodinases by RT-PCR, western-blotting, and immunohistochemistry. The activity of Deiodinases was estimated quantified rT_3 and T_3 using T_4 as a substrate. Mothers with GDM showed higher levels of TT_3 during all pregnancy, and an increased in TSH during second and third trimester, while lower concentrations of neonatal TT_4, FT_4, and TT_3; and an increased TSH level in umbilical cord blood from GDM. Placentae from GDM mothers have a higher expression and activity of Deiodinase 3, but lower Deiodinase 2, than NGT mothers. In conclusion, GDM favors high levels of TT3 during all gestation in the mother, low levels in TT4, FT4 and TT3 at the delivery in neonates, and increases deiodinase 3, but reduce deiodinase 2 expression and activity in the placenta.
Keywords: Gestational diabetes Mellitus, Thyroid hormones, placenta
Financing: FONDECYT11170710, FOVI210057

Symposium 2. **"New therapeutic strategies and molecular targets for ovarian cancer"**

1. Antitumor effects of microRNAs in different models of epithelial ovarian cancer

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MicroRNAs (miRs) are non-coding RNAs, whose function is to inhibit the translation of messenger RNAs and protein levels. In cancer, there are oncogenic and tumor suppressor miRs whose function is to change oncoproteins levels. When tumor suppressor miRs are decreased, oncogenic proteins increase.
In epitelial ovarian cancer (EOC) we found that NGF and its receptor (TRKA) increase during EOC progression. In EOC cell lines, NGF/TRKA signaling increase several oncogenic proteins such as VEGF, C-Myc, COX2, ADAM17. For this reason, we performed in sillico studies, searching for miRs that were involved with the oncogenic proteins found in EOC. Six miRs were found related with NGF/TRKA, then two of them, miR-23b and miR-145 selected because their target mRNAs of same oncogenic proteins regulated by NGF/TRKA in EOC cells. Subsequently, we evaluated the levels of miR-23b and miR-145 in EOC tissues and EOC cell lines, finding a decrease in both miRs during EOC progression and in EOC cell lines compared with non-tumor cells. Therefore, we evaluated the effect of NGF/TRKA on the expression of these two miRs in EOC cellular lines and we found that NGF decreased these miRs. Then, a reporter gene assay found that NGF decreased the expression of miR-145.
Additionally, when miR-145 and miR-23b together were overexpressed, a greater decrease in proliferation, migration and invasion was found, compared to the overexpression individually of each of the miRs in EOC cell lines. When miR-145 was stably overexpressed in EOC cell lines and grafted into immunosuppressed mice, it was found a decrease in tumor sizes. Therefore, gold nanoparticles with miR-145 were prepared to treat EOC cells in both 2D and 3D cultures, finding a decrease in viability and migration in EOC cells, as well as the number and diameter of spheroids, as well as the viability. Currently, we are evaluating the effect of both miRs on EOC cell formation in 3D cultures.
Keywords: Epithelial Ovarian Cancer, miR-145, miR-23b
Financing: Fondecyt Regular N°103061, 1071036, 1110372, 1160139 and 1220479

2. Drug repurposing and ACSL4 inhibition as a possible complementary treatment for ovarian cancer

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(2) 2 Departamento de Obstetricia y Ginecología, Facultad de Medicina norte, Universidad de Chile

Background: Epithelial ovarian cancer (EOC) is the most lethal gynecological malignancy worldwide. In advanced stages, first line treatment against EOC is cytoreductive surgery and platinum-based chemotherapy. Since cisplatin resistance is a usual phenomenon, the study of new complementary therapies is quite relevant. Acyl-CoA synthetase long chain family member 4 (ACSL-4) is involved in drug resistance in several kinds of cancers. In addition, retrospective studies have showed that common drugs as non-steroidal anti-inflammatories (NSAIDs) or metformin could decrease EOC incidence and/or mortality in different cohorts of EOC patients.
Material and methods: A2780, OV90, A2780 cisplatin-resistant (A2780-cis), and immortalized ovarian surface epithelial cell line (HOSE), were stimulated with different concentrations of metformin and diclofenac in the presence and absence of cisplatin. Additionally, we performed a characterization of ACSL-4 levels in ovarian cancer tissues and EOC cell lines. We use cell viability assays (CCK-8) to obtain dose-response curves, cell migration was evaluated using transwell® inserts and protein levels were evaluated by western-blot and immunohistochemistry. We performed three-parameter regression curves and applied Kruskal Wallis and/or Mann-Whitney test according as the case.
Results and discussion: The combined use of metformin (200 µM) plus diclofenac (50 µM) decreased the cell viability of the three EOC cell lines, compared to cisplatin alone ($p<0.05$). The combination of metformin and diclofenac impaired the migration potential of EOC cells in absence or presence of cisplatin ($p<0.05$). Additionally, EOC tissues express high levels of ACSL-4 compared to non-cancerous tissues ($p<0.05$) and the use of an ACSL-4 inhibitor decrease growth potential of A2780 cells.
Conclusion: The use of repurposing drugs (drugs used for new purposes) such as metformin and diclofenac, in therapeutically concentrations, could sensitize EOC cells to cisplatin, decreasing their cell viability and migration potential. Additionally, ACSL-4 could be an emerging therapeutic target in EOC.
Keywords: Epithelial ovarian cancer, metformin, NSAIDs
Financing: Concurso Semilla 2022 Hospital Clínico Universidad de Chile (1349/23) (M. Garrido) and FONDECYT regular 1220479 (C. Romero)

3. Development of a therapeutic strategy against cisplatin-resistant ovarian cancer cells

Manuel Valenzuela-Valderrama[1], Maria Ignacia Rubilar[1], Rosy Brito[1], Annegrett Palavecino[1], Maritza Garrido[2]
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Background: Epithelial ovarian cancer (EOC) is one of the gynecological malignancies with the highest incidence and mortality in women worldwide. It is generally diagnosed late and the development of resistance to cisplatin is usual. Arsenic trioxide (ATO) has emerged as an effective agent against cancer. In response to ATO, the nuclear factor erythroid 2–related factor 2 (Nrf2) initiates the transcription of genes necessary for antioxidant response, including those involved in GSH synthesis. Moreover, Nrf2 transcriptional activity is inhibited following RARα activation. Of note, there is evidence that RARα expression increases in advanced stages of EOC, being a marker of poor prognosis and a possible therapeutic target that has so far been poorly explored.
Methods: Human EOC (A2780, A2780-Cisp^R, OV90, and SKOV-3) and normal (HOSE) cells were exposed to different concentrations of ATO and retinoids. Drug cytotoxicity was determined by a proliferation assay (MTT), loss of clonogenic capacity (colony formation), exclusion of a vital dye (trypan blue exclusion assay), and Hoeschst 33342 staining (chromatin condensation). The changes in the expression levels of RAR α, β, γ, and the transcriptional targets of Nrf2 were evaluated by qPCR and Western-blot. Changes in GSH content were determined by colorimetric enzymatic assay. Cell migration was assessed by the Boyden chamber approach.
Results: We found that RARα is overexpressed in cisplatin-resistant cells. Furthermore, when resistant cells were treated with a RARα agonist, GSH levels decreased significantly (>85%) following Nrf2 transcriptional activity inhibition, which occurred slightly in cells that did not present resistance and maintained low levels of RARα. Interestingly, the RARa agonist synergized ATO cytotoxicity and also decreased the migration of ovarian cancer cells expressing high levels of RARα.
Conclusions: ATO treatment, combined with a specific RARα agonist, emerges as a hitherto unexplored therapeutic tool that could overcome the problem of drug resistance in EOC.
Keywords: Retinoic acid receptor, ATO, drug resistance, glutathione, ovarian cancer
Financing: CIP2021014 (M.G. and M.V., Universidad Central de Chile) and FONDECYT #1230590 (M.V.).

Symposium 3. **"Perinatal brain and systemic vascular function: the placental link with chronic adult diseases"**

1. Maternal supraphysiological hypercholesterolemia: from placental function to future cardiovascular risk

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(3) Hospital Naval, Obstetricia y Ginecología, Punta Arenas, Chile
(4) Universidad de los Andes, Facultad de Medicina, Santiago, Chile

Background. Maternal Supraphysiological Hypercholesterolemia (MSPH) is prevalent in approximately 25% of pregnancies, resulting in fetoplacental endothelial dysfunction, impaired placental lipid traffic, children fatty streaks, and higher cardiovascular disease severity later in life. Despite that, MSPH lacks clinical determination and early detection for management of its adverse effects. Aim: to assess the early identification of MSPH and its correlation with cardiovascular risk markers in early pregnancy as well as its impact in the fetus.
Methods. In a prospective cohort of 83 patients, maternal blood samples were obtained at 1-14 weeks (T1) and 35-37 weeks (T3) of gestation. A second cohort was used to test the fetal impact of MSPH. Lipid profile, apolipoprotein AI (ApoAI), apolipoprotein B (ApoB), and lipid peroxidation (MDA) levels were determined in maternal serum. Logistic regression models were performed with these variables to obtain ROC curves (specificity/sensibility for early determination of MSPH were calculated). In the second cohort, fetal aortic intima media thickness (IMT) was determined by ultrasonography at T3. Lipid profile, oxidized LDL (ox-LDL) levels, and MDA were determined in cord blood.
Results. In T1, women developing MSPH exhibit elevated levels of Apo B, Apo A, and MDA despite having normal lipid profile. The ROC curve analysis suggests that women at risk of developing MSPH in T3 can be identified at T1 with a specificity of 88% and sensitivity of 73%. Neonates from MSPH women showed similar lipid profile and IMT despite increased levels of ox-LDL and MDA compared to neonates from MPH.
Conclusion. We developed an algorithm for the early detection of MSPH. Despite presenting a normal lipid profile at T1, MSPH women and their offspring exhibit cardiovascular risk markers, suggesting pregnancy as a window for intervention.
Ethical declaration. Approved by ethical committee of U. de los Andes (CEC2021051, CEC201986).
Keywords: Pregnancy, cholesterol, cardiovascular disease
Financing: Fondecyt 1230527,1241103 and 3240476. Proyecto Interuniversitario de iniciación en investigación. Basal Funding for Scientific and Technological Center of Excelence, IMPACT, #FB210024. AM hold a PhD fellowship form the "Vicerrectoría de Investigación y Doctorados", Universidad San Sebastian.

2. Effects of perinatal hypoxia on junctional molecule involved in the blood-brain barrier permeability

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Background. The blood-brain barrier (BBB) is centrally positioned within the neurovascular unit (NVU). BBB breakdown leads to leakages of components into the cerebral parenchyma, attributed to the loss of expression of the junctional complexes impacting on BBB permeability. We have hypothesized that perinatal hypoxia modifies the permeability of the BBB. Furthermore, we will identify the molecules of junctional complexes of the BBB that are affected by perinatal hypoxia.
Methodology. 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 BBB permeability was evaluated by qPCR, western blot, and immunohistochemistry in neurovascular tissue; a non-parametric student t-test was used. Significative differences were considered when $p<0.05$.
Results. The albumin-immunopositive areas showed a significant increase in the brain parenchyma in Hx compared with Nx vessels. The expression of genes and proteins related to the neuro endothelial integrity showed a decrease in the expression of claudin 5, 3, and 12 when we compared the Nx group with the Hx group; In addition, the expression of adapter molecule was significantly lower in the Hx group compared to the Nx group.
Conclusions. PerinatalHypoxia dramatically impacts newborn 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.
Ethical declaration: All animal experimentation was approved by the Institutional Animal Care Committee (certificate 20418-MED-UCH).
Keywords: Tight junction, Neuroendothelium, Gestational Hypoxia, Perinatal hypoxia
Financing: Funding. Fondecyt de Inicio 11200798 and Fondecyt Regular 1241626

3. Metformin Impairs Pregnancy Outcomes in a Mouse Model of Maternal Hypercholesterolemia

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Background: Metformin has been suggested to improve maternal vascular dysfunction associated with pregnancy disorders; however, its use during pregnancy remains controversial. In this study, we evaluated the effects of metformin on maternal and fetoplacental outcomes in both healthy pregnant mice and a mouse model of high-cholesterol diet (HCD)-induced maternal vascular dysfunction during pregnancy.
Methods: Pregnant C57BL-6 mice were fed either a HCD or standard control diet (CD) between gestational day (GD) 13.5 and GD18.5. At GD13.5, one group of HCD and CD mice were treated with metformin (5 mg/ml) dissolved in water (4 groups in total, n=3). Mice were weight, and food and water intake were recorded. At GD18.5, females were euthanized, and placental and fetal outcomes were collected. Placentas were fixed in formalin for morphological analysis by HE staining. Results are expressed as mean±SD, and data were analyzed by two-way ANOVA followed by Sidak's post hoc testing. $P<0.05$ was considered statistically significant. Animal experiments were approved by CICUAL Facultad de Medicina, Universidad de Valparaíso (BEA 018-2024).
Results: HCD in late pregnancy reduced maternal weight gain compared to control mice (9±1 vs 3.7±2.1, $p<0.0001$), but metformin treatment decreased maternal weight either in HCD (-1±2.1) or control (8.3±2.1) groups ($p=0.0318$). No changes on water or food intake were found. The number of pups was reduced in HCD mice versus control groups (7±1.7 vs 9.7±0.6, $p=0.0304$). Metformin increased the number of reabsorptions in HCD mice, only (1.3±0.6 vs 0.3±0.6, $p=0.0339$). Fetal weight was reduced in HCD group versus control group (0.9±0.1 vs 1.2±0.07, $p<0.0001$); an effect that was exacerbated by metformin treatment. No difference in placental weight were found between groups, although fetoplacental ratio was reduced in HCD groups versus controls (9.2±1.3 vs 12.5±0.9, $p=0.0038$). Placentas from metformin-treated mice exhibited histological disorganization and an increased total vascular area, an effect that was more pronounced in those from females on a HCD.
Conclusions: Our preliminary data indicate that metformin negatively impact maternal weight during pregnancy. Female mice on a HCD treated with metformin exhibited significant illness. This study suggests that metformin has a detrimental effect on pregnancies complicated by pathophysiological hypercholesterolemia.
Keywords: pregnancy, high-cholesterol diet, metformin, fetoplacental outcomes
Financing: INICI UVA 22991, Universidad de Valparaíso.

4. Mechanosensitive mechanisms contributing to placental vascular function in health and disease

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Background: Hypoxia, inflammation and altered blood flow patterns (e.g. shear stress) are key clinical markers in fetal growth restriction (FGR), and they exert a tight control of vascular development and function across life. However, how these key stimuli interact and impose an epigenetic program on the endothelial function remains elusive.
Methods & **Results:** We analyzed transcriptional profiles of human endothelial cells to determine the effect of altered shear stress patterns, hypoxia and inflammatory conditions on Piezo1 (the main mechanosensory in endothelial cells) and mechanosensitive-related genes (MRG). In silico analyses were validated in vitro by assessing PIEZO1 transcript levels in both umbilical artery (HUAEC) and vein (HUVEC) endothelium. Transcriptional profiling showed that PIEZO1 and some MRG associated to the inflammatory response were upregulated in response to high (15 dyn/cm2) and extremely high shear stress (30 dyn/cm2) in HUVEC. Changes in PIEZO1 and inflammatory MRG were paralleled by p65 but not KLF or YAP1 transcription factors. Similarly, PIEZO1 transcript levels were upregulated by TNF-alpha (TNF-α) in diverse endothelial cell types, and pre-treatment with agents that prevent p65 translocation to the nucleus abolished PIEZO1 induction. Conversely, transcriptome analysis of FGR EC showed downregulation of several genes related to MGR (i.e. focal adhesion-PI3K-Akt). This effect was also observed in HUVEC exposed to hypoxia in vitro. Further analysis showed that both FGR (PLAEC) and hypoxia (HUVEC) were associated with decreased levels of Piezo1 and other key MRG. Analysis of Piezo1 induced relaxation and protein in the endothelium of umbilical vessels showed decreased levels in FGR umbilical veins compared with control. Downregulation of Piezo1 transcript was mimicked by deferoxamine and prevented by the HIF-1α inhibitor Syp-5.
Conclusions: Genes involved in vascular mechanosensing are regulated by hypoxia and inflammatory inputs, and changes in their expression contribute to the endothelial dysfunction observed in FGR.
Ethical declaration: Approved by CEC-UOH
Keywords: hypoxia, inflammation, shear stress, fetal vascular function
Financing: Fondecyt Regular 1220421

Symposium 4. **"Blood Brain Barrier development"**

1. Placenta-brain communication in preeclampsia. Role of extracellular vesicles in the disruption of the blood-brain barrier

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Preeclampsia (PE) is one of the leading causes of maternal mortality in low and middle-income countries (LMIC). Most maternal deaths associated with PE are related to cerebrovascular complications, which include epilepsy, stroke, or posterior reversible encephalopathy syndrome (PRES), among others. The physiopathology of cerebrovascular complications in PE remains unclear. However, we and other groups have reported disruption of the blood-brain barrier (BBB) generated by hitherto unidentified circulating factors in the maternal circulation. We recently reported that plasma and small extracellular vesicles (sEVs, 30-150 nm) derived from plasmas of women with PE disrupt the BBB. Considering that placental sEVs are elevated in PE, constituting about 10% of the circulating sEVs in plasma, we then aimed to demonstrate whether placental sEVs can disrupt the BBB. Using an *in vivo* model, we injected sEVs isolated from placentas cultured in hypoxia (sEVs-Hyp) as a proxy of what happens in PE. Results demonstrated that sEVs-Hyp can disrupt the BBB *in vivo*. However, it was unclear which factor(s) within the sEVs might be responsible for this disruption in PE: candidates such as proinflammatory cytokines or vascular modulators (such as the vascular endothelial growth factor; VEGF) have been independently suggested to play a role. More recently, we found that disruption of the BBB requires incorporating the sEVs-PE into brain endothelial cells. Also, ex vivo studies showed sEVs-Hyp high extravasation and reduced claudin 5 (CLND5) levels in the brain cortex of injected mice. Furthermore, sEVs-PE and sEVs-sHyp had higher VEGF levels than sEVs-NP and sEVs-Nor. Human brain endothelial cells exposed to sEVs-PE or sEVs-Hyp exhibited a reduction in the activation of KDR. Reduction in CLDN5 observed in cells treated with sEVs-Hyp was further enhanced in cells treated with KDR selective inhibitor. Then, sEVs-PE disrupts the BBB, an effect replicated with sEVs-Hyp, and involves reduced CLDN5 and elevated VEGF contained within these vesicles. However, our results do not support the participation of KDR activation in the downregulation of CLDN5 observed with sEVs-Hyp. These findings will improve our understanding of the pathophysiology of cerebrovascular alterations in women with PE.
Keywords: Blood-brain barrier; extracellular vesicles; preeclampsia; maternal death; placenta
Financing: Fondecyt 1240295
Acknowledgments: National and International collaborators part Leon et al., 2021 and Sandoval et al., 2024.

2. The insect Blood-brain barrier as a model for understanding development and human diseases

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The nervous system is isolated from the circulation to faithfully maintain ion and nutrient homeostasis by the blood-brain barrier (BBB). This barrier forms occluding cell-cell junctions to prevent paracellular influx of macromolecules and the infiltration of brain parenchyma by blood cells. At the same time the BBB actively controls the intake of nutrients into the brain by the action of several solute transporters. The mammalian BBB is formed by endothelial cells and its function is regulated by pericytes and astrocyte end-feet. Arthropods, such as *Drosophila melanogaster*, are characterised by an open circulatory system and a glial BBB. The subperineurial glial cells cover the entire nervous system and generate a paracellular diffusion barrier by the formation of septate junctions. The perineurial glial cells generate a second layer, which participates in the metabolic support of the brain. How the *Drosophila* BBB is established during development and prevents the progression of neurodegenerative diseases, is not completely understood.
We are currently studying the role of subperineurial and perineurial glia during the development of the central nervous system. Using genetically encoded tools, we have shown that subperineurial glia projects long membrane processes through the brain neuropil, suggesting a direct connection between them. These processes are controlled by the GPCR signalling. Furthermore, we have analysed the effect of perineurial glial ablation during development. We found that perineurial layer are required for the normal establishment of occluding junctions in the subperineurial layer. Finally, we are analysing the effect of the dysfunction of the BBB on the accumulation of amyloid-beta in an Alzheimer´s disease model.
Keywords: Drosophila melanogaster, Glial cells, Blood-brain barrier
Financing: Fondecyt Iniciación en Investigación N°11230539

3. Cholesterol transport in the formation of the blood-brain barrier

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Background: Formation of the brain vasculature involves morphogenesis and barriergenesis of blood vessels. These processes are regulated by multiple signaling pathways, including NOTCH, and endothelial metabolic balance. Cholesterol metabolism has been proposed to regulate NOTCH signaling; thus, we hypothesized that cholesterol can regulate brain vascularization.
Methods: We have employed cell cultures and mice models to evaluate the role of cholesterol in brain endothelial cell biology. In vitro, methyl-β-cyclodextrin (CD) was used to remove cholesterol from a brain endothelium cell line (bEnd3). In vivo, we have evaluated vascularization in mice lacking either SR-B1 or ABCA1, proteins mediating cholesterol uptake or efflux, respectively. We explored the effects of these manipulations using immunofluorescence and RNA sequencing.
Results: Treatment with CD resulted in a 2.5-fold reduction of cholesterol in bEnd3 cells, which was associated with increased wound closure in the scratch assay (+35%; $p<0.05$, t-test); we did not observe changes in proliferation as assessed by EdU incorporation. There were massive changes in gene expression in CD-treated cells: cholesterol synthesis was upregulated, whereas efflux was downregulated; genes involved in tight-junction formation were upregulated; the classic NOTCH target Hey1 was downregulated. In mice lacking the cholesterol importer SR-B1, we observed no major vascular defects at embryonic day (E)14.5, E18.5 or in adults. However, transcriptomic analysis of isolated vascular fragments showed a trend opposite of CD-treated cells. Finally, embryos lacking the cholesterol efflux pump ABCA1 showed reduced vascularization of the brain at E18.5 (30% less vascular coverage; $p<0.05$, t-test) and leakage of endogenous IgG into the developing cortex.
Conclusions: Our studies have begun to reveal an important role of cholesterol in the regulation of the developing brain vasculature transcriptome and morphogenesis.
Keywords: Cholesterol, Blood-brain barrier
Financing: ANID Fondecyt de Iniciación 11240017
Acknowledgments: Approved by IACUC at U. de los Andes (CEC2024006)

Symposium 5. **"Unconventional models in reproduction and development: expanding horizons using zebrafish, under-studied invertebrates and amphibians"**

1. Studying germline evolution and polyspermy blockage beyond animal model systems

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Reproduction is a fundamental characteristic of living organisms, essential for the survival and continuation of species. In animals that reproduce sexually, the germline represents a unique cell type crucial for reproduction and heredity. Germ cells, along with the mature gametes they produce, are the carriers of heritable genetic and epigenetic information passed to the next generation. Despite the importance of germ cells in transmitting hereditary information across generations, the mechanisms of their segregation in animals remain not fully understood. Once in their terminally differentiated states, gametes fuse during fertilization to form a totipotent diploid zygote. To ensure that only a single sperm fertilizes an egg, animals have developed various mechanisms to prevent polyspermy (fertilization by multiple sperm), including transient versus slow blocking and egg membrane versus coat blocking. However, the molecular determinants involved in blocking polyspermy are still not well known. Our understanding of germline segregation and polyspermy prevention in animals is largely derived from studies in prominent model organisms, including vertebrates such as fish, frogs, zebrafish, and nice, as well as highly derived invertebrates like *Drosophila* and *Caenorhabditis*. Here, I discuss recent findings on germline segregation and polyspermy prevention in scientifically neglected yet highly informative animal taxa. These findings offer new insights into various stages of germline specification, from germline stem cells to differentiated oocytes, and trace the molecular repertoire and evolutionary dynamics of germline-specific genes throughout evolution. By expanding the sampling of animal taxa and employing a phylogenetically comparative approach, we can significantly advance our understanding of stem cells, pluripotency mechanisms, and reproductive success in animals.

Keywords: germline segregation, polyspermy blockage, cnidarians, mollusks, sea urchins
Financing: Funding: Fondecyt 1220708; Fondequip EQM200056; eLife-Mesocosm; FOVI230192

2. Maternal genetics of the oocyte-to-embryo transition

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The transition from oocyte to embryo represents a critical developmental phase marked by a complex interplay of molecular processes across the oocyte, egg, and early zygote stages. This period of transcriptional silence is driven by precisely timed functions of maternally-loaded gene products, accumulated during oogenesis. Despite their essential roles, the specific molecular identities of these factors remain only partially understood. Phenomics, as an emerging genome-wide discipline, offers a powerful approach to link gene sequences with functional traits. By utilizing zebrafish, a highly tractable vertebrate model, we provide a robust platform for exploring maternal genotype-phenotype relationships and their implications for human reproduction and associated diseases. Our research employs a comprehensive approach, integrating genetic screens with phenotyping, to identify key genes that govern the oocyte-to-embryo transition in zebrafish. In this symposium, we highlight the roles of two critical factors: the conserved maternal Ap5m1 protein, which regulates yolk granule maturation—essential for nutrient provision and immune defense during early development—and the Mgat1a factor, which modulates the secretory pathway, thereby influencing egg activation. These genetic mutants serve as valuable models for dissecting the molecular and cellular mechanisms underlying this essential developmental process in vertebrates. Our discoveries not only provide a detailed resource of phenomics data but also offer functional tools to explore the evolutionary pathways of maternal factors. Furthermore, these insights significantly enhance our understanding of the developmental strategies crucial for reproductive success across diverse metazoan species.
Keywords: Meternal genes, Ap5m1, Mgat1a, zebrafish, Phenomics
Acknowledgments: Proyecto Fondef IDeA I+D ID23I10264

3. Zebrafish as a model to study the teratogenic potential of drugs and pollutants

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Every year thousands of new chemical compounds are produced and released by one way or another to the environment, and their impact on embryonic development is generally unknown.
Zebrafish has been widely proposed and proved to be an extraordinarily model in several research areas in biology and biotechnology, including developmental biology, reproductive toxicology and drug discovery. This, due to its several advantages including the external development, high fecundity, transparency, and the genetic similarity to mammals. A wide variety of bioassays to assess the toxicity and bioactive properties of chemical compounds, have been design and validated in the last decades, positioning the zebrafish as one of the most versatile biological models, with direct application in the pharmaceutical and chemical industry, and with bioethical advantages over mammalian models.
Diverse versions of bioassays with zebrafish have been proved to successfully predict the embryotoxic and teratogenic effects of chemicals in mammals at relevant concentrations. However, several relevant aspects remain unclear, especially those related to real scenarios in which the risk of exposures are to mixtures of chemical, no to isolated compounds, or the presences of different biological barriers that protects the human and the fish embryos.
In this symposium, I will introduce the advantages (and disadvantages) of the zebrafish for studies of developmental toxicology and the relevance of the diverse tools available in this model. These approaches can be helpful to assess the impact on embryonic development of specific chemical agents or to unveil their modes of action, but also to detect their presence on aquatic ecosystems or to study different biological activities with potentially clinical interest.
Keywords: zebrafish, embryotoxicity, teratogen, bioassay
Financing: Danio Biotechnologies, SpA. VRID-UdeC 2023000931INT

4. Participation of ATP and Glutamate signaling during Neural Tube formation in the chordate Xenopus laevis

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Neurulation is a crucial process in the formation of the central nervous system (CNS) in all chordates, which initiates with the folding and fusion of the neural plate, leading to the generation of the Neural Tube and subsequent development of CNS structures like the brain. Several known paracrine signals like WNT and FGF participate significantly in Neural Tube formation wherein others pathways such us purinergic and glutamatergic signaling has been described more recently. All these signals are required for the control of cellular process necessary during Neurulation like change in cell shape, migration and proliferation. Genetic and environmental factors like the presence of anti-seizure medications (ASM) interfere with the neurulation process promoting neural tube defects (NTDs) and others latter and more complex alterations in neural function like autism spectrum disorders (ASD). Our laboratory investigate specifically the presence and functionality of purinergic and glutamatergic signaling during Neurulation process and the consequences of interfere with these signals in the establishment and functionality of the CNS using Xenopus laevis model.
We found that glutamatergic signaling is active through NMDA ionotropic receptor, modifying intracellular Ca2+ dynamics allowing the oriented cell migration and proliferation. In addition we demonstrated the presence and functionality of glutaminase 1 (GLS1, glutamate synthesis enzyme). Although protein expression levels remains constant, the catalytic activity of GLS1 increases significantly (~66%) between early (stage 12) and middle to late (stages 14–19) neurulation. Then, using 6-diazo-5-oxo-L-norleucine a competitive inhibitor of glutamine-depend enzymes, the activity of the GLS1 was reduced (~36%) and the occurrence of NTDs was induced significantly.
Associated to purinergic signaling and using molecular and cellular techniques, we demonstrate the expression and functionality of connexin hemichannels, including Cx46 and Cx32, which are associated with the release of ATP. Furthermore, applications of FGF2 and/or changes in intracellular redox potentials (DTT), well known HCs-Cxs modulators, transiently regulated the ATP release in our model. Importantly, the blockade of HCs-Cxs by carbenoxolone (CBX) and enoxolone (ENX) reduced ATP release with a concomitant formation of NTDs.
This information contribute to elucidate whether these drugs would have the ability to affect critical signaling during relevant periods of neural development.
Keywords: neurulation, purinergic signaling, glutamate, antiseizure medication (ASM), Xenopus
Financing: Fondecyt 1231038

NEW MEMBERS TALKS

1. Enrique Guzmán (Symposium 1, nº3) and **2. Tamara Sáez** (Symposium 3, nº3)

3. Placenta and maternal mental health during COVID-19 pandemic

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(4) U. de Concepción, Depto. de Bioquímica Clínica e Inmunología, Facultad de Farmacia.
(5) U. del Desarrollo, Facultad de Psicología, Concepción, Chile
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Introduction: The COVID-19 pandemic has impacted many aspects of health and society worldwide. A vulnerable group during the pandemic was pregnant women, who were considered to have potentiated risk factors. In physiological pregnancy, maternal systems have several changes and adaptations to support fetal development. These changes involve regulations of cardiovascular, respiratory, and immunologic systems, among others, which SARS-CoV-2 could severely alter.
Objective: To analyse the evidence about the effects of the COVID-19 pandemic on placenta function and maternal mental health.
Methods: Review of the literature, combined with experimental analysis of placenta from COVID-19 patients. Healthy controls (n=6) and gestational COVID-19 cases (n=17) were obtained during the pandemic period, before massive vaccination (Ethics committee certification: CEC-SSC 20-11-60). Placental samples were analyzed by histology and immunohistochemistry to evaluate vascular alterations, inflammation, and oxidative stress. Results: Our experimental findings showed placental inflammation, oxidative and nitrosative stress, and significative alterations of vasculo-syncytial structure in placental villi in COVID-19 cases, especially in pregnant women with severe COVID-19. On the other hand, the review of the published evidence showed significant increases in anxiety and depression in pregnant women, with higher levels in the Latin-American population compared with European.
Discussion: The conditions of Latin American pregnant women during the COVID-19 pandemic were harder than other populations in Europe or North America, which was reflected in higher rates of perinatal anxiety and depression. Still, there is no direct correlation between maternal mental health and placental dysfunction, but there is evidence about prenatal anxiety/depression and plasma inflammatory markers that could impact the placenta.
Keywords: COVID-19, placenta, mental health, pregnancy
Financing: FONDECYT Regular 1241905
Acknowledgments: To the patients and personnel of Hospital Clínico Regional Guillermo Grant Benavente (Concepción, Chile)

4. The absence of the zona pellucida modifies the molecular signals produced by domestic cat blastocysts

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Background: Domestic cats are an important model for the development of assisted reproductive techniques (ARTs) in endangered felids. It has been postulated that an intact zona pellucida (ZP) is crucial for implantation of in vitro produced felid embryos. However, the possible regulatory role of the ZP in this process is poorly understood. The objective of this study was to evaluate the effect of the in vitro development without the ZP on the molecular signals and the implantation capacity of domestic cat blastocysts.
Methods: two groups were created: 1) domestic cat blastocysts generated by IVF (Zona intact, ZI group) and 2) domestic cat blastocysts generated by IVF and cultured without the zona pellucida (Zona free, ZF group). The in vitro development and the implantation rate were evaluated. The gene expression (pluripotency, trophectoderm and apoptosis markers) and miRNAs expression were evaluated by RT-qPCR at the blastocyst stage. The Wilcoxon non-parametric test was used ($p < 0.05$). Finally, a proteomic analysis to identify differentially expressed proteins (DEPs) between ZF and ZI blastocysts was performed.
Results: No differences were observed in the in vitro development at the blastocysts stage between groups. However, the ZF blastocysts were not capable of implant after embryo transfer. Furthermore, ZF blastocysts had a reduced expression of the pluripotency markers SOX2 and NANOG, and an overexpression of the trophectoderm markers YAP1 and EOMES. Additionally, ZF blastocysts had overexpression of miR-21, miR-25, miR-29 and miR-199 and a reduced expression of miR-96. Finally, the ZF blastocysts had 22 upregulated and 20 downregulated proteins compared to ZI blastocysts.
Conclusions: The culture of domestic cat embryos without the ZP did not affect the in vitro development. However, ZF blastocysts were no capable of implant, which might be related to their altered gene and protein expression, indicating a possible regulatory role of the ZP.
Keywords: in vitro development, gene expression, felid embryos, microRNA, proteomic analysis
Financing: ANID Fondecyt Postdoctorado 3200352.
Acknowledgments: Ethical approval: Comité de Bioética, Facultad de Ciencias Veterinarias, Universidad de Concepción (CBE—08-20).

5. Exposure to PM2.5 from wood smoke induces placental vascular changes and reduces foetal size

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During gestation, maternal blood flow to the umbilical cord and placenta increases, facilitating efficient nutrient absorption, waste elimination, and effective gas exchange for the developing fetus. However, the effects of exposure to wood smoke during this period on these processes are unknown. We hypothesize that exposure to PM2.5, primarily sourced from wood combustion for home heating, affects placental vascular morphophysiology and fetal size. We used exposure chambers that received either filtered or unfiltered air. Female rats were exposed to PM2.5 during pre-gestational and/or gestational stages. Twenty-one days post-fertilization, placentas were collected via cesarean section. In these placentas, oxygen diffusion capacity was measured, and the expression of angiogenic factors was analyzed using qPCR and immunohistochemistry. In groups exposed to PM2.5 during pre-gestational and/or gestational stages, a decrease in fetal weight, crown-rump length, theoretical and specific diffusion capacity, and an increase in HIF-1α expression were observed. In groups exposed exclusively to PM2.5 during the pre-gestational stage, there was an increase in the expression of placental genes Flt-1, Kdr, and PIGF. Additionally, in the placental labyrinth region, the expression of angiogenic factors was elevated. Changes in angiogenesis and angiogenic factors reflect adaptations to hypoxia, impacting fetal growth and oxygen supply. In conclusion, this study demonstrates that exposure to PM2.5, emitted from wood smoke, in both pre-gestational and gestational stages, affects fetal development and placental health. This underscores the importance of addressing air pollution in areas with high levels of wood smoke, which poses a significant health risk to pregnant women and their fetuses.
Keywords: placenta, hypoxia, pollution, PM2.5, angiogénesis
Financing: Fondecyt N°11200775

SHORT ORAL TALKS

1. Expression of thyroid hormone receptors in human trophoblast HTR-8/SVneo cell line.

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Background: Trophoblasts cells are the main cell type present in human placenta and are responsible for the implantation processes and embryonic development. Thyroid hormone signaling via thyroid hormone receptors is essential for growth regulation and cellular differentiation process. HTR-8/Svneo cell line is widely used to study trophoblast functions. However, thyroid genomic and no-genomic hormone receptors (i.e. Thyroid hormone receptor (THR) α, THRβ1 and αV/β3-integrin) have not been fully characterized.
Objective: To determine the presence of thyroid hormone receptors expressed in HTR-8/Svneo cell line.
Method: HTR-8/Svneo cells were seeded on poly-D-lysine coated coverslips. After 24 hours incubation (37°C, 5% CO_2), cells were fixed with 4% paraformaldehyde and permeabilized with 0,5% Triton-X. After blocking with 4% bovine serum albumin, cells were incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibody for 4 hours at room temperature; nuclei were counterstained with DAPI. After washing three times, cells were mounted onto slide glasses with antifade Prolongue™ Gold. Cells were analyzed using a Leica SP8 LIGHTNING confocal spectral microscope.
Results: Confocal images analysis revealed a positive expression were suggesting that THRα and THRβ1 are expressed and predominantly localized in nucleus and cytoplasm, while αV/β3-integrin is expressed and localized in cytoplasm of human trophoblast HTR-8/Svneo cell line.
Conclusion: Thyroid hormone receptors THRα, THRβ1 and αV/β3-integrin are expressed in human trophoblast HTR-8/Svneo cell line. These results suggest a crucial role of thyroid hormones in placental function, opening new research possibilities regarding their potential involvement in gestational pathologies.
Keywords: thyroid hormone receptor, thyroid hormone, trophoblast
Financing: None

2. Pannexin 1 channels activity in human trophoblast HTR8/Svneo cells

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Background. Pannexins are large-pore, nonselective membrane channels linked to purinergic signaling, including inflammatory responses. This family consists of three isoforms, Panx1, Panx2, and Panx3, which have been detected in first-trimester placental explants. Nevertheless, the role of Panx channels in the placental organ remains largely unexplored. This study aims to assess Panx1 activity in the HTR8/Svneo trophoblast cell line.
Methods. Panx1 channel activity was evaluated using a dye uptake assay. Cells were incubated for 10 minutes with DAPI (5 µM) under three experimental conditions: a control (uptake solution, pH 7.4), a positive stimulus (alkaline uptake solution, pH 8.5), and a positive stimulus with Panx1 blocker (100 µM carbenoxolone). Subsequently, cells were washed, fixed with 4% PFA, and DAPI fluorescence (excitation 358 nm, emission 461 nm) was quantified by epifluorescence microscopy. The images obtained were analyzed using ImageJ and GraphPad Prism software programs.
Results. Exposure to alkaline solution increased DAPI uptake in HTR8/Svneo cells (29358.3 ± 1616.0 A.U. vs. 9331.6 ± 728.2 A.U.), a response that was reduced by 100 µM carbenoxolone (11543.8 A.U. ± 503.1 A.U.), a blocker of Panx1 channels.
Conclusions. Our preliminary findings suggest that the trophoblast cell line exhibits Panx1-like activity. Characterization of the placental Panx1 channel may identify it as a critical pathway for ATP release in the placenta, potentially playing a significant role in placental vascular function.
Keywords: Pannexin 1, Trophoblast, Placenta

3. The maternal factor Krang: a new player in reproduction

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Background: In most animals, fertilization is strictly monospermic. Fertilization by more than one sperm, a condition known as polyspermy, is lethal. To prevent this, eggs employ mechanisms such as the cortical reaction, which involves the exocytosis of cortical granules (CGs) into the perivitelline space. This reaction hardens the zona pellucida in mammals or the chorion in fish, thereby blocking further sperm entry. For this process to occur, CGs must be synthesized, translocated to the oocyte cortex, and anchored to the plasma membrane to release their contents upon fertilization. These processes rely on maternal factors stored in the oocyte during oogenesis. However, the molecular mechanisms governing CG biology remain largely unknown.
Methods: We identified the zebrafish *krang* mutant in a forward genetic screen (CEBB 818-2020). Chorion elevation was quantified by measuring the egg-to-chorion index. CG exocytosis was tracked in eggs by using MPA staining. Sectioned whole ovaries were stained to analyze CG biosynthesis and translocation. *krang* RNA and protein subcellular localization was examined in oocytes using fluorescent *in situ* hybridization and immunofluorescence, respectively.
Results: Eggs from mutant females display altered chorion elevation and perivitelline space formation upon egg activation, due to impaired CG translocation dynamics to the oocyte cortex during oocyte maturation. The mutation impacts the highly conserved *kiaa0513* gene, resulting in a predicted protein of 24 aberrant amino acids. The localization of Krang transcripts and protein in oocytes is associated with CGs in a punctuated pattern during mid-oogenesis, suggesting that it is part of a ribonucleoproteic complex and its RNA is awaiting a spatially localized translation.
Conclusions: Our results indicate that the zebrafish maternal-effect *krang* mutant display defects in CG translocation and exocytosis, allowing us to provide insights into this novel protein´s function in animal reproduction and shed light on the maternal genetic program essential for preventing polyspermy in vertebrates.

Keywords: polyspermy, cortical granules, Krang.
Preferred type of presentation: oral presentation.
Funding: ANID 21232350.

4. TSH reduces T4 uptake in human trophoblast HTR-8/SVneo

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Background. During the first trimester of pregnancy, the mother gives T4 hormone to the fetus through placenta, particularly in syncytiotrophoblast. TSH is a regulator of T4 levels in the mother, but its role in placenta, and as a regulator of T4 uptake is unknown. However, high levels of TSH in pregnancy have been associated with negative outcomes for both the mother and the fetus.
Methods. HTR-8/SVneo cells were incubated in different concentrations of TSH (0, 1, 5, 10 and 20 mIU/L) for 24 hours. Subsequently, we applied a 100 nM T4 solution for 15 minutes at 37°C and 4°C. Then, we added a 0.68 M persulfate solution and incubated at 95°C for 1 hour. T4 uptake was evaluated by Sandell-Kolthoff reaction. For this, we added the same amount of the liquid of the plates, 25 mM ceric solution and 25 mM arsenious solution and measured the absorbance at 415 nm in a microplate reader. Results were expressed in folds of changes.
Results. The T4 uptake at TSH 1 mIU/L increased approximately 1.6-fold with respect to control. However, at 5 mIU/L, T4 uptake was reduced around 60%, but TSH values over 10 mIU/L abolish T4 uptake in trophoblast. These results indicated that TSH reduces T4 uptake in HTR-8/SVneo cells. In fact, IC50 of TSH on T4 uptake was 4.89 mIU/L.
Conclusions. The increase of TSH levels decreases the uptake of T4 in HTR-8/SVneo cells, blocking it at concentrations greater than 10 mIU/L. This may lead to the development of new treatments for hypothyroidism in pregnancy, directed to lower TSH levels rather than increasing T4 levels.
Keywords: TSH, T4 uptake, Placenta
Financing: ANID FOVI230114
Acknowledgments: I would like to thank Professor Guzmán for his encouragement and support.

5. Impact of PM2.5 Emitted from Wood Smoke on GLUT-1 and SVCT-2 Expression in Rat Placenta

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Background: During gestation, hypoxia is necessary for vascular development. However, chronic hypoxia alters placental morphology, affecting oxygen and nutrient transport, such as glucose, and inducing oxidative stress. In this context, antioxidant protection is essential for fetal development. Vitamin C plays a regulatory role in maintaining oxidative balance during pregnancy. We hypothesize that exposure to PM2.5 from wood smoke may induce oxidative imbalance that overwhelms the body's defense mechanisms, altering placental function and glucose transport, and contributing to reduced fetal size.
Methods: Sprague-Dawley rats (G2) were exposed to PM2.5 from wood burning smoke during the pregestational and/or gestational periods. They were kept in chambers with filtered air (n=24;FA;control) and unfiltered air (n=24;NFA). Exhibition location: Temuco, La Araucanía Region. Placentas were obtained by cesarean section at 21 days after fertilization, and the expression of glucose transporter (GLUT1;1:400) and vitamin C transporter (SVCT2;1:1000) was assessed by immunofluorescence. Images were analyzed with StrataQuest software. Nested ANOVA tests were performed.
Results: Exposure to PM2.5 during the pregestational and/or gestational periods significantly affects the expression of GLUT-1 and SVCT-2 transporters in the labyrinth zone of the placenta. Nested ANOVA results show statistically significant differences in the area and fluorescence intensity of these transporters across different exposure groups ($Pr(>F) = 0$). Gestational exposure was associated with a reduction in the area and intensity of GLUT-1 in cells exposed exclusively during the gestational period ($p<0.001$). For SVCT-2, the area was smaller in cells exposed exclusively during the gestational period ($p<0.001$), while the intensity increased in those exposed during the pregestational period ($p<0.001$).
C**onclusion**: These findings suggest that PM2.5 exposure may reduce the placenta's ability to transport glucose and vitamin C, compromising energy supply and antioxidant protection to the fetus. However, an adaptive response in the placenta to increase antioxidant capacity during gestation was observed.
Keywords: PM2.5, GLUT-1, SVCT-2
Financing: Fondecyt Iniciación 11200775 [Paulo Salinas]
Acknowledgments: Bioethics committee: All rat experiments were conducted in accordance with the UFRO Scientific Ethics Committee (Law-122/20)

6. Potential Therapeutic Effects of Rosemary Extract in Endometriosis: In vitro Antioxidant and Anti-Proliferative Activities

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Background: Natural therapeutic alternatives are being explored in endometriosis research due to the limitations of current treatments, which are relatively ineffective, often associated with significant side effects, and do not prevent disease recurrence. This study was conducted to characterize an ethanolic rosemary extract (RE) and evaluate its therapeutic potential in in vitro models of endometriosis.
Methods: The total phenolic content was determined using the Folin-Ciocalteu method, and the main active compounds, carnosic acid (CA) and rosmarinic acid (RA), were quantified using high-performance liquid chromatography (HPLC). The antioxidant properties of the extract were assessed using DPPH and ABTS radical scavenging capacity assays, as well as the ferric reducing antioxidant power (FRAP) assay. The effects of various concentrations of RE on cell viability were examined in two human endometrial cell lines—stromal (t-HESC) and epithelial (ECC-1)—as well as in an endometriotic cell line (12-z). Additionally, the impact of RE on cell migration was assessed in T-HESC and 12-z cells.
Results: The phenolic content of RE was found to be 18.3 ± 1.83 µg EAG/g, with 37.2 µg CA/g and 3.968 µg RA/g. The extract exhibited significant antioxidant activity, with DPPH values of 35.36 ± 4.13 mM Trolox equivalent (TE), ABTS values of 38.45 ± 0.92 mM TE, and FRAP values of 43.13 ± 0.96 mM TE. Furthermore, RE was found to significantly inhibit cell viability in ECC-1 at 5 mg/mL, in t-HESC at 8 mg/mL, and in 12-z at 7 mg/mL, while also reducing cell migration in 12-z and T-HESC cells.
Conclusion: This study highlights the potential of ethanolic RE as a natural treatment for endometriosis, demonstrating significant antioxidant activity and effective inhibition of cell viability and migration in endometriosis models; however, more clinical research is required to fully validate its efficacy and safety.
Keywords: Endometrium, Carnosic acid, Rosmarinic acid, Treatment, Potential
Financing: This work was supported by Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (ANPCYT, PICT 2019 Start Up-00037 and PICT 2021-338 to GM).

7. Modulation of deiodinase 3 expression and activity by triiodothyronine in trophoblast cells

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Background: Thyroid hormones are essential for placental and foetus development, transported to the fetus through the placenta, where trophoblastic cells are the main cell type. Key thyroid hormones include thyroxine (T4), which is present in the bloodstream, but lacks biological activity, and triiodothyronine (T3). The regulation of extracellular and intracellular levels of these hormones, are mediated by DIO3 enzymes, responsible for inactivating thyroid hormones, by converting T4 to rT3 intracellularly. Overexpression of DIO3 is frequently observed in pathological conditions, including gestational diabetes, in which, maternal T3 levels are high and DIO3 expression in trophoblast cells increases. However, it is unknown if this increase in DIO3 expression and activity in the placenta is due to elevated maternal serum levels.
Methods: The HTR8/Svneo trophoblast cell line was incubated at 37°C with increasing concentrations of triiodothyronine (0-10) and extracted at a range of time between 1-48 hours. DIO3 mRNA expression was measured by qPCR, and protein expression was evaluated by western blot.
Results: DIO3 mRNA and protein levels increased 2.2-fold significantly in HTR8/Svneo cells in the presence of T3 after 12 hours if incubation, with a significant reduction to 0.7-fold in the presence of T3 after 48 hours of incubation.
Conclusions: T3 increases DIO3 expression in human trophoblast. These results are similar to those observed in placentas from pregnancies with gestational diabetes, suggesting that increased maternal T3 may explain the heightened expression and activity of DIO3.
Keywords: Placenta, DIO3, Triiodothyronine
Financing: Funding. FOVI230114

8. Involvement of MAPK in the regulation of insulin-induced thyroid hormone transporters in trophoblasts.

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Background. The transport of thyroid hormones (THT) from the mother to the fetus ensures proper fetal development during gestation. For this purpose, there are 3 families of THT in placental tissue: Monocarboxylates transporters (MCT), Amino Acid Transporters (LAT) and Organic Anion Transporter Polypeptides (OATP). Pre-pregnancy conditions such as obesity generate pathological hyperinsulinemia during the first trimester of gestation, where insulin regulates the expression of thyroid hormone transporters in human trophoblast cells (HTR8/SVneo), however, it is not known whether mitogen-activated protein kinases (MAPK) are involved in insulin regulation.
Aim. To evaluate the involvement of MAPK on insulin-induced THT expression in the HTR8/SVneo cell line.
Methods. Immunocytochemistry was performed to evaluate the expression of MCT8, MCT10, LAT1, LAT2, OATP1A2 and OATP4A1 in the HTR8/SVneo cell line. Trophoblasts were incubated with insulin and MAPK inhibitor (PD98059) for 24 hours to assess mRNA and protein expression by qPCR and Western blot, respectively. In addition, thyroxine (T4) uptake in trophoblasts was studied by the Sandell-Kolthoff technique.
Results. Expression of MCT8, MCT10, LAT1, LAT2, OATP1A2 and OATP4A1 was observed in HTR8/SVneo trophoblasts in the absence and presence of insulin. In addition, increased OATP4A1 expression was obtained under hyperinsulinemic conditions ($p<0.05$) along with increased T4 uptake ($p<0.05$). Finally, the increase in OATP4A1 mRNA by insulin was blocked in the presence of PD98059 ($p<0.05$).
Conclusion. The upregulation of OATP4A1 transporter expression by insulin in HTR8/SVeno is dependent on the MAPK pathway. This study is important for understanding insulin-mediated effects on fetal development and long-term complications in the newborn.
Keywords: Insulin, thyroid hormone, placenta.
Financing: Funding: ANID-FOVI 210057 (EG-G).

9. Relationship between perinatal outcomes and a polymorphism of adiponectin (rs2241766) in pregnant women in Concepcion, Chile.

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Background: During pregnancy, a prodiabetogenic state occurs that can lead to hyperglycemia, meaning elevated blood glucose levels. This increase in glucose heightens the risk of perinatal complications. Adiponectin, a protein that regulates insulin sensitivity, plays a significant role in this process. However, SNP rs2241766 is associated with a decrease in adiponectin synthesis, which leads to increased insulin resistance. This uncontrolled resistance contributes to hyperglycemia and, consequently, a higher risk of complications during pregnancy.
Objective: To associate perinatal outcomes with the polymorphism rs2241766 in Chilean women.
Method: Pregnant women were recruited from CESFAMs in Concepción. The selection criteria included: adult women, Chilean, and with no history of diabetes. DNA samples were extracted from blood samples. GDM was diagnosed using an PTGO. Finally, genotyping was performed using PCR-RFLP to identify risk alleles (TG/GG) or their absence (TT).
Results: There is a significant difference between first trimester blood glucose levels and PTGO results between the NGT and GDM groups. A Pearson correlation was performed to observe the presence of risk alleles and alterations in clinical parameters. A relationship was found between risk alleles and altered blood glucose levels in the third trimester (r:0.467, p:0.033). Additionally, negative correlations were observed between risk alleles and systolic blood pressure (r: -0.34, p:0.02), TSH levels (r: -0.359, p:0.02), and T3 levels (r:0.352, p:0.02). Subsequently, a linear regression was performed to evaluate causality, and the following results were obtained: for glucose level in the third trimester (r: 0.22, p: 0.03), systolic blood pressure (r: 0.12, p: 0.02), TSH level (r: 0.13, p: 0.01), and T3 (r: 0.13, p: 0.02).
Conclusion: The presence of risk alleles demonstrates an alteration in glucose levels in the third trimester of pregnancy, as well as a decrease in TSH and T3 levels. These effects may increase the risk of developing perinatal complications.
Keyworks: Perinatal outcomes, rs2241766, Chilean woman
Funding: FONDECYT 11170710
Ethical declaration: Approved by the Scientific Ethics Committee of the Concepción Health Service (Code CEC 23-2017-20) and the Ethics Committee of the University of Concepción.

POSTER PRESENTATIONS

Characterization of sEV from menstrual fluid from multiparous women and those with previous preeclampsia

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Background. Preeclampsia (PE) is a pregnancy complication that affects 5% of all pregnant women and one of the main causes of adverse pregnancy outcomes. The main strategies proposed for early screening of women at risk of developing PE are the recognition of specific maternal risk factors and the use of multiple biomarkers that require highly expensive assays. Here we propose to characterize small extracellular vesicles (sEV) from menstrual fluid from women with previous uncomplicated pregnancies and women whose previous pregnancy was complicated by preeclampsia as a potential source of inexpensive biomarkers for early prediction of PE.
Methods. Multiparous women with a previous uncomplicated pregnancy (n=6) and women with a history of preeclampsia (n=3) were recruited up to 12 months after delivery and a sample of menstrual fluid was self-collected by the participants. Menstrual fluid small extracellular vesicles were isolated by serial centrifugations and subsequently characterized in terms of size and concentration using the Nanoparticle Tracking Analysis. Populations of CD63, CD81 and CD9 surface markers were determined by flow cytometry, and sEV total RNA content was analyzed by Next Generation sequencing. Confidential informed consent was signed by all patients.
Results: No differences were observed in menstrual fluid-derived sEV from women who have had PE and multiparous women with previous healthy pregnancies in terms of size, concentration, or surface exosomal markers. Regarding total RNA content, 1065 genes were found significantly downregulated and 1019 were found significantly upregulated in PE patients, compared to controls. Moreover, a unique signature of lncRNAs differentially contained in menstrual fluid-derived sEV from PE and controls was identified (97 downregulated vs 44 upregulated lncRNAs in PE vs Controls, $p<0.05$).

Conclusions: This study characterized the sEV obtained from menstrual fluid from previous uncomplicated pregnancies and from women with a previous history of preeclampsia, providing baseline data and information on EVs arrived from this unique source as a potential biological source to identify preconceptional biomarkers of PE, particularly lncRNAs.
Keywords: Small Extracellular Vesicles, Biomarker, lncRNAs, Preeclampsia
Financing: ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024; Fondecyt Regular #1230932, #1201851, #1241103, FONDECYT Postdoctorado #3230201; ANID-Doctorado Nacional/2023 #21230458.

Effects of WNT-inhibition and Activin A on development competence of Bovine Haploid Parthenogenetic Embryos

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Background. Haploid embryonic stem cell lines (hESCs) have advantages in biological research due to their differentiation potential and phenotype being the same as their genotype. However, most bovine HPEs (bHPEs) undergo developmental arrest which precludes their use for deriving hESCs. Therefore, this study aimed to investigate whether supplementing the culture medium with a GSK3B inhibitor (CHIR99021) or Activin A (AA) affects the developmental competence of bHPEs.
Methods. In vitro mature oocytes were activated with ionomycin (5 µM) for 5 min followed by cycloheximide (CHX) for 5 h to induce haploid parthenogenesis. Diploid parthenogenetic embryos (DPE) were included as control by activation with 6-DMAP instead of CHX. Treatments with CHIR99021 (3 µM) and AA (20 ng/mL) were performed from Day 5 onward (5-6 morulas/group) using a serum-free medium (SFM). Culture in 0.001% DMSO was considered as vehicle control. Embryo development was assessed at 72 h (cleavage), 120 h (morula), and 192 h (blastocyst). Morphological quality, immunostaining of pluripotency markers (CDX2, SOX2, GATA2, and NANOG), and DNA fragmentation were analyzed at the blastocyst stage. Binomial data sets were analyzed using Fisher's test and a p value< 0.05 was considered significant.
Results. The results showed that the SFCM did not affect the developmental competence or cell allocation of DPE. Although there was a tendency (p=0.106) for a higher proportion of morulas that developed into a blastocyst (blastulation) in bHPEs supplemented with AA (77%) compared to the control DMSO group (55%), all groups of bHPEs showed similar blastulation (range: 55%-77%), similar levels of pluripotency as well as DNA fragmentation (p>0.05).
Conclusion. These data show that GSK3B inhibition or AA supplementation does not affect the competence and embryonic pluripotency of bHPEs. Further studies will be focused on evaluating the effects of these small molecules on the derivation efficiency of bovine hESCs.
Keywords: haploidy, embryo, ivf
Financing: ANID – FONDECYT INICIACION 11230091, UNIVERSIDAD DE LA FRONTERA GI24-0039.
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Effects of sperm capacitation with bicarbonate and polyvinyl-alcohol on in vitro outcomes of equine ICSI

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Background. Intracytoplasmic sperm injection (ICSI) in horses is currently employed for clinical and commercial uses, but the protocol could be optimized to improve its efficiency. We have hypothesized that the induction of changes associated with sperm capacitation prior to injection would positively impact the developmental potential of equine zygotes generated by ICSI. Thus, this study evaluated the effects of sperm treatment with bicarbonate and polyvinyl alcohol (capacitating conditions, CAP) on developmental features initially following heterologous ICSI on bovine oocytes and subsequently in homologous ICSI employing equine oocytes.
Methods. Equine sperm were incubated for 45 min in CAP or control (no-capacitating, NC) conditions and subsequently used for ICSI. Quantitative data sets were analyzed using one-way ANOVA and Tukey test. Binomial data sets were analyzed by Fisher test. Differences were considered significant at $P < 0.05$. Oocyte collection, piezo-assisted ICSI, and embryo culture were performed according to standard protocols.
Results. Following heterologous ICSI, the results showed that oocyte activation assessed by rates of pronuclear formation (NC: 65% (26/40), CAP: 75% (30/40)) and cleavage (NC: 72.82% (75/103), CAP: 83.18% (89/107)), were similar between groups. However, the levels of 5-methylcytosine (associated with gene repression) at the 8-cell stage, were higher in CAP than in NC conditions (15 vs. 9 Arbitrary units of Mean Fluorescence Intensity, respectively). Similarly, after homologous ICSI, the proportion of cleavage (NC: 79.71% (55/69), CAP: 85.71% (60/70)), the blastocyst rate (NC:16% (11/69), CAP: 21% (15/70)), blastocyst size (average= 150 µm) and their total cell number (average= 220 cells) were similar between groups.
Conclusion. These results suggest that the specific capacitation condition evaluated in the present study does not impact the *in vitro* competence of equine embryos generated by ICSI. Further studies will be focused on the implications of differential levels of DNA methylation on the early development of equine zygotes.
Keywords: ICSI, sperm, equine, embryo
Financing: Funding. ANID – FONDECYT INICIACION 11230091; ANPCYT (FONCyT, Argentina—Préstamo BID), Grant/Award Numbers: PICT 2018 01681, PICT 2021-I-A-00129)
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Expression of hCAT-1 and eNOS in placenta from pregnant women with SARS-CoV-2 infection

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Background: The endothelial dysfunction, probably related to the perturbation of the metabolism of nitric oxide (NO), has been observed during the infection with SARS-CoV-2. For NO synthesis, the expression of the human cationic amino acid transporter, hCAT-1, and the enzyme endothelial NO synthase, eNOS, are fundamental. Additionally, has been observed vascular alteration in placental samples from pregnant women with SARS-CoV-2 infection, but the expression of hCAT-1 and eNOS in these patients has not been documented.
Objective: To determine the expression of hCAT-1 and eNOS in placental samples from pregnant women with SARS-CoV-2 infection.
Methods: Placental samples from pregnant women, with (severe or mild symptoms of COVID-19) and without SARS-CoV-2 infection were obtained during COVID-19 pandemic, approved by CEC from Servicio de Salud del Biobío (CEC-SSC: 20-11-60). Total ARN were obtained for hCAT-1 amplification by RT-PCR. Besides, tissue microarray samples stained with H/E for structure observation and immunohistochemistry for eNOS were analyzed.
Results: In histological samples, we observed a decrease in the developed placental structures, also appearing fibrin deposits and higher levels of macrophages. The expression of hCAT-1 changed between negative patients and positive for the infection, being significant the increase in the chorionic plate samples from severe cases ($p=0.0381$). Also, we observed a higher expression of eNOS in the severe patients by immunohistochemistry ($p=0.0181$).
Conclusions: In the samples positive to the SARS-CoV-2 infection, we observed alterations in placental structures, with an increase in Hofbauer cells (macrophages) and a decrease in vasculosyncitial membranes. In patients with severe symptomatology, it's also observed a significant increase in expression of hCAT-1 and eNOS. These alterations could be due to a placental response to inflammation induced by severe COVID-19.
Keywords: Placenta, hCAT-1, eNOS
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Effect of endometriosomes from endometrial cell lines on quality and development of IVF bovine embryos

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Background. In vitro fertilization (IVF) has a low efficiency in terms of embryo quality and development, being the in vitro culture media a crucial factor. Therefore, the aim of this work was to evaluate the effect of small extracellular vesicles (sEVs), isolated from endometrial cell lines, in the in vitro culture medium and on the development and quality of bovine embryos.
Methods. Matured oocytes were fertilized in vitro and the resulting zygotes were cultured. On day 3, endometriosomes from the CBR-BESC-UpEx and CBR-BEEC-UpEx cell lines were added. Embryo quality was assessed at day 7 by immunofluorescence with specific antibodies. To assess the internalization of sEVs, these were stained and incubated with embryos, and then visualized under a confocal microscope. The expression of genes involved in embryonic development was performed by RNA extraction, followed by cDNA synthesis, concluding with RT-qPCR, using housekeeping genes for normalization.
Results. As for cleavage and blastocyst rate, embryos treated with the CBR-BEEC-UpEx sEVs show statistically significant differences with respect to the control. In terms of embryo quality, a potentially higher number of cells in the inner cell mass with both types of endometriosomes is observed; however, the results are inconclusive due to the resolution of the images. On the other hand, a high internalization of endometriosomes is evident in the embryos, with CBR-BEEC-UpEx embryos showing a more uniform distribution. Finally, the analysis of relative gene expression reveals significant differences in CDX2 and CASP3 genes involved in cell differentiation and cell death.
Conclusions. The results obtained suggest that a low expression of the CDX2 gene may be attributed to the activation of early regulatory mechanisms. These results will allow a better understanding of the function of sEVs in early embryogenesis, as well as the improvement of IVF in cattle.
Keywords: IVF, Bovine embryos, Small extracellular vesicles (sEVs)

Financing: Funding. DI-UFRO Project DI20-0062, FONDECYT Project #1201166, and the National Doctoral Scholarship ANID #21191434, Chile. Ethical declaration: The project has been approved for implementation by the Scientific Ethics Committee (CEC) of the Universidad de La Frontera (N°125/20).

Lack of SR-B1 does not affect the development of the cerebral vasculature of mouse embryos

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Background: The membrane receptor *SR-B1* binds to HDL cholesterol to mediate the direct transfer of cholesterol between the lipoprotein and the cell. Cholesterol metabolism is important for modulating BBB function in adulthood in healthy and diseased animal models. Since *SR-B1* is involved in cholesterol uptake, we hypothesized that the lack of this protein would reduce vascular development of the embryonic brain, impairing BBB formation.
Methods: An adult mouse model was used in which the female lacks the SR-B1 receptor and heterozygous parents were mated. Mice were maintained in the animal facility at Universidad de los Andes. Embryos were dissected on embryonic day 14.5 (E14.5) and genotyped by conventional PCR with primers specific for wild-type and knockout alleles. Fetal heads were fixed in 4% PFA overnight at 4°C and preserved in 30% sucrose. Coronal sections of 20 microns were obtained with a cryostat. Brains were fluorescently stained with isolectin B4 and CD31 antibodies to reveal endothelial cells. We determined brain vascular coverage and branch points density as an approximation of angiogenesis.
Results: In histological sections with immunofluorescent staining for CD31 and IB4, blood vessel coverage was determined in a control group (WT and HET embryos) and SR-B1 KO embryos (6.4±0.9 vs 6.7±0.6% coverage, respectively; p=0.24, t-test, N=6 samples per group). Blood vessel branching, defined as number of bifurcations of blood vessels, was also similar in control and SR-B1 KO embryos (30.6±3.2 vs 33.4±5.2 branches/section respectively; p=0.45, t-test, N=6 samples per group). There were no significant differences for the coverage and vascular branching in SR-B1 KO mouse embryos.
Conclusions: We observed similar vascular coverage and branching in the brains of embryos lacking SR-B1, suggesting normal angiogenesis at E14.5 in this model. Future studies will evaluate the integrity of the BBB in this model.
Keywords: SR-B1, Isolectin B4 (IB4), CD31.
Financing: Funding: ANID FONDECYT de Iniciación #11240017
Acknowledgments: Ethical declaration: Approved by IACUC from U. de los Andes (CEC2024006).

Deficiency of MUL1 in mice results in developmental defects and impaired growth

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Background: MUL1 is a mitochondrial protein involved in mitochondrial dynamics. MUL1 inhibits cell growth, induces apoptosis, and modulates the immune system. In this context, MUL1 has been related to cardiovascular, inflammatory, neurological, metabolic, and cancer, however, little is known concerning its role during fetal development. This study aimed to characterize the effect of MUL1 deficiency [heterozygous (HET) and knockout (KO) mice] during development and the phenotypic cardiometabolic changes resulting from impaired MUL1 expression.
Methods: C57BL6N/ HET, WT, and KO MUL1 mice were bred and genotyped by conventional PCR at 21 days after birth and 12 weeks of age. The following parameters were assessed: body weight, mouse size, glucose tolerance curve in HET mice, and cardiac hypertrophy markers. In addition, a survival analysis (Kaplan-Meyer) was performed. Structural histological analysis of cardiomyocytes and muscle was perfomed at 21 days and 12 weeks. Data were analyzed using non-parametric t-tests and one-way ANOVA.
Results: There was a reduced rate of fetal survival and effective crossover percentage in MUL1 deficient mice (HET-HET is 64%, KO-KO 0%, and KO-HET 75%) compared to wild-type offspring. A significant decrease in body weight and size was observed since early postnatal days. Kaplan-Meyer analysis showed lower survival of C57BL6N KO animals compared to HET and WT animals. Histological analysis of the heart and muscle showed differences in cell size in KO mice compared to the other experimental groups.
Conclusions: Total MUL1 deficiency in mice negatively influences fetal survival, body growth, and weight. However, no significant changes in metabolic parameters were observed under basal conditions measured in KO and HET mice compared to WT mice.
Keywords: mitochondria, cardiac, Mul1
Financing: Funding: Fondecyt Regular 1220421

Micro-RNA 21-5p protects neonatal rat cardiomyocytes from hypoxia-induced dysfunction by enhancing survival pathways

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Background: Micro-RNA-21-5p is highly expressed in the cardiovascular system, however, there is limited evidence concerning its role in fetal life. Here, we characterized the transcriptional effect of miR-21-5p upregulation in neonatal rat cardiomyocytes (NRC) exposed to hypoxia in vitro.
Methods: NRC were obtained from 3-day-old rat pups by enzymatic dissociation, transfected with 30nM miR-21-5p mimic, and exposed to normoxia or hypoxia (1%O2) for 6 hours. Apoptotic cells were detected by TUNEL staining. Transcriptional profiling was performed using long-read sequencing and differentially expressed genes (DEG) were determined by comparing the conditions tested [normoxia (Nx), normoxia with miR-21-5p (Nx-miR21), hypoxia (Hx), and hypoxia with miR-21-5p (Hx-miR21)]. We considered fold-changes of ±1.2 (FDR) as statistically significant ($P<0.05$). DEG were further investigated using functional enrichment analysis.
Results: NRC exposed to hypoxia showed increased Tunel-positive staining, an effect reverted by miR-21-5p. A total of 774 DEG were found among all the conditions relative to Nx, of which about 300 were differentially regulated between Hx vs. Nx-miR21 and Hx-miR21. Several hypoxia-related DEG were targeted by miR-21-5p. The transcriptional profile of Hx cells was enriched in pathways related to cardiovascular dysfunction and cell death. Conversely, there was an enrichment of genes involved in protection against cell death (e.g. NFkB) and fluid shear stress response (e.g. mitophagy) in Hx- miR21 treated cells.
Conclusions: Hypoxia promotes a transcriptional profile highlighting dysfunction and apoptosis in neonatal cardiomyocytes. Upregulation of miR-21-5p protects appears cardioprotective by stimulating cell survival pathways.
Keywords: hypoxia, cardiomyocyte, miRNAs
Financing: Funding: Fondecyt Regular 1220421

H233L and H398P phospholipase Cζ mutations impair both phosphatidylinositol bisphosphate and calcium ion binding

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Background: Infertility affects 30 million men worldwide. Several causes have been described, and genetic factors are responsible for ~30% of the cases. Mutations in a sperm-specific phospholipase, phospholipase C zeta (PLCζ), have been related to male infertility. PLCζ is transferred to the egg during the sperm-egg fusion, and it hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to produce inositol 1,4,5-triphosphate (IP_3) and Diacylglycerol (DAG). IP_3 binds to the IP_3 receptor 1 (IP3R1), located in the Endoplasmic Reticulum, ER, triggering efflux of Ca^{2+} from the ER. Periodic increases of intracellular Ca^{2+} $[Ca^{2+}]_i$, Ca^{2+} oscillations, underlie egg activation, critical for the egg-to-embryo transition. The exchange of histidine for leucine in position 233 (H233L) and histidine for proline in the position 398 (H398P), causes infertility in humans. These mutations impair Ca^{2+} oscillations and egg activation. The PLCζ structural impact of these mutations on the PLCζ function have not been described. Using full-atom molecular dynamics simulations we assess the impact of H233L and H398P on PLCζ structure and PIP_2 and Ca^{2+} binding.
Methods: AlphaFold server was used to predict the hPLCζ (Uniprot code: Q86YW0). CHARMM-GUI was used to mutate PLCζ and build the systems. MD simulations were performed with AMBER. The protein-ligand affinity energy was computed utilizing the end-point Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) method of AMBER22.
Results: Analysis of PLCζ-PIP2 interactions revealed that the H233L and H398P mutations significantly reduce the number of hydrogen bonds, salt bridges, and Van der Waals interactions with PIP2. Additionally, these mutations increase the binding free energy. Furthermore, the H233L and H398P mutations impair Ca^{2+} binding and induce a displacement of both PIP2 and Ca^{2+}.

Conclusions: The mutations H233L and H398P resulted in fewer hydrogen bonds and salt bridges between PLCζ and PIP2, as well as reduced total contacts and binding energy, suggesting a destabilization of the PLCζ-PIP2 complex. Our results also indicate that the mutations H233L and H398P modify the Ca^{2+} ion binding. Thus, both mutations favor PIP2 and Ca^{2+} mispositioning, preventing PIP2 hydrolysis.

Keywords: Infertility, Phospholipase C zeta, Calcium, egg activation, PIP2

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The potential role of menstrual mesenchymal stem cells in the etiopathology of endometriosis

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Background: Endometriosis (EM) is a gynecological disorder of unknown etiology, characterized by the presence of endometrial cells outside their normal uterine location. It affects approximately 10% of women of reproductive age globally and is associated with chronic pain and infertility. Retrograde menstruation is the most accepted hypothesis for EM, thus characterizing menstrual derived-mesenchymal stem cells and their role in the establishment of ectopic endometriotic lesions during menstrual backflow could broaden the understanding of endometriosis' origins and development.
Methods: Mesenchymal stem cells were isolated from menstrual fluid of women with EM (MenSC-EM) or surgical confirmation of absence of abdominal and pelvic endometriosis (MenSC-Control). MenSC were immunophenotyped by flow cytometry. Clonogecity and transmigration capacity were evaluated using colony forming assay and transwell assays, respectively. For decidualization, MenSC were submitted to 10 nM estradiol, 1µM progesterone and 0.5 mM cAMP for 3 days.
Results: MenSC-C and MenSC-EM expressed surface mesenchymal cell markers CD90, (99.68% vs 99.55%), CD73 (100% vs 99.96%), CD44 (99.95% vs 99.93%), and CD105 (99.05% vs 99.53%) (C vs EM). No significant differences were observed in clonogenic capacity following 10-14 days of culturing 25, 50, 100, and 150 cells. Decidualization potential, assessed by the mRNA expression of IGFBP1 and PRL showed no significant differences between MenSC-C and MenSC-EM ($p=0.1$, Mann-Whitney test). MenSC-EM tend to have greater migratory capacity compared to MenSC-C (1.75 times higher). MenSC-EM tend to have higher expression of surface proteins EpCAM and N-cadherin compared to MenSC-C (34.7% vs. 14.3%, $p=0.057$, Mann-Whitney test).
Conclusions: MenSC were successfully isolated and characterized from both control and EM samples, confirmed by the presence of mesenchymal surface markers. MenSC-EM exhibited a potential increase in migration capacity, though larger sample sizes are needed to confirm. Elevated EpCAM and N-cadherin levels in MenSC-EM could suggest greater adhesion potential, possibly contributing to endometriotic lesion formation.
Keywords: Endometriosis, Mesenchymal Stem Cells, EpCAM

Financing: ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024; Fondecyt Regular #1230932, U. de los Andes FAIN #202201; and Subdirección de Capital Humano/Becas Doctorado Nacional/2023-#21230458.

Exposure to particulate matter from wood smoke on cellular proliferation and ovarian apoptosis in rats

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Background. Studies have shown that exposure to PM2.5 has adverse effects on biological systems. According to our review, there is a lack of research focused on the effects of PM2.5 derived from wood smoke on the reproductive system (Villarroel et al., 2024). The aim of the study was to describe the changes induced by exposure to PM2.5 from wood smoke in the ovary, integrating the dynamics of cell growth and death, to demonstrate potential impacts on female reproductive health.
Methods. A controlled experiment was conducted in a city (38°44'59.4"S 72°37'07.8"W) with high levels of air pollution from wood combustion, exposing nulliparous G2 Sprague-Dawley rats to filtered (AF; n=12) and unfiltered (ANF; n=12) air conditions, thereby mimicking human exposure to PM2.5. In primordial, primary, and secondary follicular cells, as well as ovarian granulosa cells, cellular proliferation (Novus Biologicals, 110-89719; 1:400) and apoptotic indices (ApopTag, S7101, Merck, Germany) were measured. Data were analyzed using GraphPad Software (San Diego, CA).
Results. The results indicate that proliferation in granulosa cells did not differ between groups in primordial ($p = 0.5802$), primary ($p = 0.9042$), and secondary follicles ($p = 0.4656$). However, a decrease in proliferation was observed in antral follicles in the ANF group ($p = 0.0203$). Regarding the apoptotic index, no differences were detected between groups.
Conclusions. Exposure to PM2.5 from wood smoke affects cell proliferation in antral follicles, while it does not affect apoptosis in different types of follicles. Understanding the affected mechanisms is vital for developing strategies to protect reproductive health in exposed populations.
Keywords: cell proliferation, apoptosis, pollution, particulate matter 2.5, KI67 protein
Financing: FONDECYT #11200775 (Paulo Salinas)

Prenatal exposure to particulate matter (PM 2.5) from wood smoke on fetal endochondral ossification

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Background. Exposure to air pollution during gestation has been associated with adverse outcomes. We hypothesize a placental hypoxic scenario related to PM2.5 exposure, potentially impacting growth due to hypoperfusion of the growth plate in developing bone. The aim of this study was to determine the effect of gestational exposure to PM2.5 on endochondral ossification.
Methods. Rats were exposed to filtered air (FA; control; n=24) or unfiltered air (NFA; n=24) during the pregestational and/or gestational stages (FA/FA, FA/NFA, NFA/FA, and NFA/NFA groups) in exposure chambers (38°44'59.4"S 72°37'07.8"W). Postfertilization (21 days), fetuses were obtained via cesarean section. Endochondral ossification was assessed using radiography and clearing techniques. The structure and expression of growth factor (GF)-related proteins (BMP-2, TGF-β, and FGF) and Hypoxia-Inducible Factor (HIF1-α) were studied in the growth plate of the tibia.
Results. The FA/FA group showed trabeculae with osteocytes in a homogeneous, anastomosed bone matrix, with osteoblasts and osteogenic cells at the edges (++). In the NFA/NFA group, trabeculae with sufficient thickness for visible osteocytes,homogeneity, and medullary space with osteoblasts were observed (+++). The NFA/FA group shared characteristics with FA/FA, presenting osteocytes and osteoblasts (+). The FA/NFA group showed thick trabeculae, osteocytes in the matrix, and few visible osteoblasts (+). All groups had 50-90% of their blood vessels organized. Regarding the presence of osteoclasts, they were scarce in FA/FA, moderate in FA/NFA and NFA/FA, and abundant in NFA/NFA. The NFA/NFA group showed reduced TGF-β ($p=0.025$) and increased FGF ($p=0.0084$) compared to the control (FA/FA). There were no differences in HIF1-α ($p=0.1531$), BMP-2 ($p=0.5810$), or chondrocyte density ($p=0.2609$).
Conclusions. This study highlights the detrimental effects of exposure to wood smoke during the pregestational and gestational stages on endochondral ossification. These effects manifest as structural changes and alterations in the expression of growth factors related to mineral metabolism, leading to reduced bone formation, osteoblastic differentiation, and osteoid matrix synthesis. These findings suggest adaptations in response to exposure that impact fetal size development. Our findings underscore the significance of the environment during early gestational stages on long-term bone health, emphasizing the need to address wood smoke as a risk factor.
Keywords: endochondral ossification, pollution, particulate matter 2.5, growth factors, HIF1a
Financing: FONDECYT #11200775 (Paulo Salinas)

Küzyñ, the role of placenta for Mapuche culture in the context of Chilean Health System

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Background: Among the ancestral practices of the Mapuche people in relation to birth, the buried of the placenta stands out. For this reason, technical note 189 has been implemented, which consists of a protocol for the delivery of the placenta to Mapuche women. A problem in the implementation of intercultural health strategies is the unilateral definition of health policies, without equitably considering the knowledge of the communities.
Objective: To collect experiences about practices associated with the placenta in the Mapuche people and to determine the knowledge of health teams about technical note 189.
Methods: Mixed qualitative study, using in-depth interviews for Mapuche women and semi-structured interviews for the health team. Individual interviews of midwifes were recorded, as well as conversations with Mapuche women from Biobio and La Araucanía regions (Ethics certificate code CEC-SSC 22-03-05).
Results: Concerning the health team (n=13), in general, the midwife's pointed out that they know the technical note 189 and were informed that this protocol can be applied when the pregnant women solicit it. However, all of them indicated that they never received information about intercultural health, nor training about the application of the technical note 189. About the birth experiences of Mapuche women (n=5), the interviewees state that the treatment and respect for their culture improve greatly in hospitals that have an intercultural facilitator and that have implemented the birth plan. It is also noted that to carry out the burial of the placenta, the well-being of the family and community is required, as well as available ancestral territory.
Conclusions: The health team don't have enough information about the Mapuche culture associated with birth and placenta. Among Mapuche women, although the practices associated with the placenta may vary between communities, there is agreement that the burial of the placenta is relevant for the conservation of Mapuche culture.
Keywords: Mapuche, placenta, intercultural
Financing: Intercultural grant UCO 1995 (UdeC) "Protocolo de entrega de placenta para pueblo mapuche: hacia una visión intercultural de la atención del parto"
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Characterization of extracellular vesicles derived from breast milk of women with hypercholesterolemia

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Background: High total cholesterol (HTC) levels are the main cause of cardiovascular disease. Women during lactation (L) can present this condition, when breast milk (BM) is produced to nurse their infants. BM provides complete newborn´s nutrition during the first months of life, and among other compounds, contains extracellular vesicles (EVs). Their role in BM is related to inflammatory pathogenic contexts of the infant. However, it is unknown if during the L, BM-EVs and their cargos from HC-women are altered in quantity and cargo, presenting a pro-inflammatory profile.
Methods: Serum (to categorize NTC<200mg/dL or HTC, >200mg/dL) and BM-samples were obtained from women between 1st-5th lactation month. 6ml of BM were used to measure macronutrients and 10ml to obtain BM-EVs by ultracentrifugation. BM-EVs were characterized measuring proteins enriched in EVs; Alix, and TSG101, and proteins present in BM; Casein, and Lactoferrin. The size and concentration of BM-EVs were determined by nanoparticle tracking analysis (NTA), and the morphology by Transmission Electron Microscopy (TEM), respectively.
Results: 27.2% of recruited women presented HTC. BM-macronutrients were similar between NTC and HTC-women. BM-EVs from HTC-group showed increased casein abundance (203.9%, *p<0.05), and decreased lactoferrin abundance (55.9%, *p<0.05). BM-EVs derived from NTC and HTC present the typical proteins associated to EVs, and respect to NTC Alix and TSG101 decreased (60.2%, *p<0.05 and 41%, *p<0.05) in HTC-group. The percentage of particles <200nm were similar in NTC and HTC-groups. The concentration of particles present in BM-EVs from HTC-women were decreased (60.2%, p=0.05). No differences were found in the morphology and size distribution of BM-EVs between groups.
Conclusions: BM-EVs from HTC-women present alteration in their cargo and concentration. Further studies are needed to evaluate if these particles have a pro-inflammatory role.
Ethical Declaration: The study has ethical approval from Central Metropolitan Health.
Keywords: Extracellular vesicles, Breast Milk, High Total Cholesterol
Financing: Funding: ANID grant SA77210098 (to SC-D), FONDECYT 1230983 (to MV-G).
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Design of genetic construct to immortalize bovine endometrial cells by transfection with the TAg-SV40 gene

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Background. Endometriosomes secreted by bovine endometrial cells cultured in vitro have great potential to improve in vitro fertilization (IVF) in terms of embryonic development and quality. However, after approximately 8 passages, these cells quickly enter senescence, abandoning their original phenotype. The objective of this research was to design a construct that allows BESC and BEEC endometrial cells to be immortalized, to increase their number of possible passages.
Methods. The plasmid pTMB was constructed, containing a blasticidin resistance gene and a polycistron that encodes the fluorescent protein mCherry and the TAg-SV40 protein, capable of immortalizing animal cells. Through a series of high-fidelity PCR amplification procedures, enzymatic restrictions, DNA ligations, and purification of DNA bands from electrophoresis gels, unnecessary sequences were removed and the various components of pTMB were ligated. The pTMB was subcloned into competent Escherichia coli DH5α bacteria, selecting the colonies transformed with ampicillin, which were subsequently validated by Colony-PCR. Colonies containing pTMB were cultured in liquid LB medium for 16 hours at 37°C and shaking at 180 RPM. The plasmid was purified from the bacterial culture using Miniprep kit. Finally, pTMB was used to transfect and immortalize endometrial cells.
Results. Colony-PCR validated the correct construction of the pTMB. The transfected cells showed resistance to blasticidin and expressed the TAg-mCherry polycistron. Immortalization was confirmed by the presence of the mCherry protein and subculture of the cells until passage 10, without showing apparent morphological changes.
Conclusions. In this work, 2 immortalized bovine endometrial endometriosome producing cell lines were generated, with potential use in improving IVF. In addition, pTMB can be used in the immortalization of other animal cell types with biomedical or research interests.
Keywords: Cell immortalization, Improvement of IVF, Genetic engineering.

Financing: Funding. This research was funded by the DI-UFRO Project DI20-0062, FONDECYT Project #1201166, and the National Doctoral Scholarship ANID #21191434, Chile. Ethical declaration: The project has been approved for implementation by the Scientific Ethics Committee (CEC) of the Universidad de La Frontera (N°125/20).
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Skull ossification divergence in Xenopus frogs: a trade-off between bone deposition and ostecyte formation?

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Background: Understanding the genetic bases of phenotypic divergence is a daunting challenge in developmental biology. To tackle this issue, we have compared skull development and transcriptome between two related frog species, *Xenopustropicalis* (*Xt*) and *Xenopus laevis* (*Xl*).
Methods: To quantify cranial growth rate, we applied pulses of Alizarin red and Calcein green in larvae from stages NF54-60. The density of osteocytic lacunae and their characteristics were quantified by SEM. Osteoblasts were observed by fluorescent microscopy. Collagen fibres and bone thickness were visualized with Two-photon microscopy at stage NF60. Gene expression was examined by RNA-Seq performed on undifferentiated osteogenic mesenchyme and on differentiated osteoblasts.
Results: Both species show opposite trends in their developmental ossification rate, as bone deposition slows down for *Xt* while it dramatically accelerates for *Xl*. In *Xt*, the bone has greater thickness and contains more osteocyte lacunae than *Xl*. Amongst the genes that are much more strongly expressed in *Xl*, we identify *col2a1* and *col18a1* (Coding for collagenous proteins not usually associated to the bone matrix), *pcolce* (Involved in Collagen maturation) and *sytl4* (Involved in exocytosis). By contrast, the expression of *hif1a*, *hif2a*, *phospho1* and *enpp1* in *Xt* is consistent with a higher rate of osteocyte differentiation in this species.
Conclusions: We propose that the larger and more rapidly expanding *Xl* larval skull requires a higher demand in surface matrix deposition than *Xt*. By contrast, the *Xt* smaller skull favours local bone thickening and osteocyte differentiation. Hence, we identify genes involved in skull growth in *Xl*, and other genes involved in osteocyte differentiation in *Xt*, thereby suggesting that these two processes are antagonistic. Our study contributes to bridging the gap between genotype and phenotype during skeletogenesis.
Keywords: Xenopus, Skull development, Frontoparietal bone
Financing: FONDECY 1190926.

Evaluation of cryopreservation tolerance of Atlantic Salmon (Salmo salar) sperm under different broodstock dietary compositions.

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Background. Developing new diets for fish is a constant challenge in aquaculture. Substituting fishmeal and oils with animal derivatives, terrestrial plants, and microalgae aims to improve sperm quality and cryopreservation tolerance in fish reproduction. In this study, the effect of different diets on the tolerance of Atlantic salmon sperm to cryopreservation was evaluated.
Methods. Four experimental groups were established: Diet Group1: exclusively marine-origin protein and lipid ingredients., Diet Group2: substituted 65% of the proteins and 51% of the lipids with terrestrial animal and plant sources, Diet Groups3 and4: commercial diets. The broodstock males were fed for a period of six months. Sperm quality evaluation was performed on sexually mature males; samples were extracted by testicular maceration and stored at 4°C. Subsequently, they were cryopreserved in Cortland medium supplemented with 1.3 M DMSO and 0.3 M glucose in 0.5 ml straws (IMV), frozen in liquid nitrogen vapors, and thawed in a thermostatically controlled bath. The concentration was adjusted to $2x10^9$ sperm/mL for further analysis of sperm physiology.
Results. The results showed that the sperm from Diet Groups 1 and 2 had a higher percentage of viability and plasma membrane integrity (PMI: 73±5.1% / 82±8.2%), Mitochondrial membrane potential (MMP: 54±7.1% / 51±8.1%), motility rate (MR: 66±5.9% / 60±5.8%) and lower production of cytoplasmic superoxide anion (O_2^-: 31±4.2% / 36±7.1%) and DNA fragmentation (DNAfrag: 2.3±0.5% / 3.1±0.7%) compared to Diet Groups 3 and 4 (PMI: 64±5.7% / 65±6.1%; MMP: 45±5.8% / 32±4.8%; MR: 55±6.8% / 53±8.2%; O_2^-: 53±6.9% / 44±7.1%; DNAfrag: 5.3±0.8% / 4.1±0.5%, respectively; $p<0.05$)

Conclusions. The partial replacement of ingredients of marine origin in fishmeal and fish oil, with ingredients of terrestrial animal and plant origin, affects the sperm quality of non-frozen intratesticular samples, considering motility, the integrity of the plasma membrane, the potential for mitochondrial membrane and oxidative stress parameters.

Keywords: Cryopreservation, diet composition, sperm quality
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Decoding the novel function from Krang gene in animal secretory vesicle biology

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Cortical reaction is essential in preventing polyspermy. During this process, cortical granules (CGs) exocytose their content into the perivitelline space, promoting the expansion of the zona pellucida (ZP) in mammals (chorion in fish) and blocking further sperm entry. For this reaction to occur, CGs undergo several stages, involving the function of different proteins. However, many of these remain unidentified. The zebrafish *krang* mutant, isolated through a genetic screen, exhibits defects in early embryonic development, due to impaired chorion expansion, presumably caused by fewer CGs reaching the oocyte cortex compared to wild type. Genetic analysis revealed a 35-nucleotide deletion at the 3´-end of exon in *krang* gene, leading to the loss of the protein´s functionality. The localization of *krang* transcript in oocytes suggests a specific association with CGs. The maternal Krang protein expresses during mid oogenesis stages and localized in the vicinity of CGs in pre-maturing oocytes, as studied by immunodetection. In addition, altered expression of Krang in the human brain is linked to Alzheimer´s disease and schizophrenia, supporting the hypothesis that Krang functions in exocytic systems. Immunofluorescence localization analysis also shows Krang in the otolithic vesicle and neural tube of 24 and 48 hours post-fertilization zebrafish larvae and a punctate pattern in the central nervous system. Understanding the Krang´s function in reproduction may not only provide insights on cortical reaction but also allowsexploring the cellular origins of these neuropathologies, potentially informing new treatment approaches.
Keywords: Krang, oogenesis, central nervous system

Machine Learning Models to Predict the Probability of Pregnancy in High Complexity Treatments of IVF

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Background: Clinical pregnancy rates in in vitro fertilization (IVF) cycles are influenced by infertile couples and embryo development factors, making accurate pregnancy prediction challenging. We aim to develop a machine learning model (ML) to improve the prediction of pregnancy success in IVF treatments.
Methods: Data from fresh IVF cycles performed at IDIMI from 2015-2019 were analyzed. The parameters included maternal age, BMI, antral follicle count (AFC), anti-Müllerian hormone levels, cause of infertility, duration of stimulation, number of mature oocytes retrieved, blastulation rate, and number of transferred embryos. Four machine learning models were developed and optimized: Gradient Boosting, Random Forest, Support Vector Machine (SVM), and XGBoost. These models were evaluated using stratified group k-fold cross-validation. Performance metrics included accuracy, F1 score, precision, recall, specificity, and ROC-AUC.
Results: XGBoost model achieved an AUC ROC of 0.7308, with an accuracy of 67.09%, an F1 score of 0.6389, precision of 0.6970, recall of 0.5897, and specificity of 0.7500. Gradient Boosting model had an AUC ROC of 0.6724, accuracy of 60.76%, F1 score of 0.5974, precision of 0.6053, recall of 0.5897, and specificity of 0.6250. Random Forest model achieved an AUC ROC of 0.7519, accuracy of 69.62%, F1 score of 0.6757, precision of 0.7143, recall of 0.6410, and specificity of 0.7500. SVM model had an AUC ROC of 0.7301, accuracy of 65.82%, F1 score of 0.6667, precision of 0.6429, recall of 0.6923, and specificity of 0.6250. SHAP analysis highlighted the significance of blastulation rate, age, AFC, and other clinical parameters in predicting treatment outcomes. These results show that Random Forest model can effectively predict IVF outcomes, with the highest performance compared to the other models.
Conclusions: ML, particularly the Random Forest model, is a robust tool for guiding medical professionals in decision-making and may hold promise for future research and application in reproductive medicine.
Keywords: IVF, machine learning, predictive modeling
Financing: Non funding study
Acknowledgments: Ethical Declaration: This study was approved by the San Borja Arriarán ethical committee.

Cav3.2 calcium channels function during oocyte maturation and egg activation

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Background: The acquisition of meiotic competence (oocyte maturation) comprises the progress of a Prophase I of Meiosis I immature oocyte, Germinal Vesicle, GV, to a Metaphase II of Meiosis II (MII) egg. The oocyte maturation involves several processes including the migration of cortical granules (CGs) to the egg cortex in preparation for fertilization. Cortical granules (CGs) are vesicles whose exocytosis is essential for preventing polyspermy. GC exocytosis depends on increases of intracellular Ca^{2+}.The mechanisms underlying Ca^{2+} influx supporting oocyte maturation and activation have not been fully characterized. The Ca^{2+} channel Cav3.2 has been reported to function during oocyte maturation contributing to the filling of the Ca^{2+} store, however, the role of Cav3.2 in CGs distribution, and extrusion of the second polar body has not yet been studied.
Methods: 7-15 weeks WT and Cav3.2 knockout (TKO) females were superovulated with intraperitoneal (i.p.) injections of PMSG followed 48 h later by i.p. injection of hCG. Eggs were stained with DyLight Lectin 649 (20 µg/ml) and DAPI Vectashield, then imaged using a Leica confocal microscope. Eggs were activated by SrCl2 (10mM) incubation (2h) at 37°C and 5% CO2.
Results: TKO eggs have reduced size in comparison to WT eggs (TKO=73.31µm vs. WT=74.17µm; *p=0,0428). TKO eggs showed significantly increased lectin fluorescence intensity on the cortical area than WT (**p=0.0086). SrCl2 activation in WT and TKO eggs show a similar rate reaching 2 cell stage after 24h post incubation with SrCl2 (~70%), however, the release of the second polar body was delayed in TKO in comparison to WT eggs (46.7% in TKO vs 82.3% of WT at 2 h post SrCl2 incubation).
Conclusion: Our data suggest that Cav3.2 function is essential to set the eggs size and CGs distribution. Moreover, Cav3.2 modulates the timing of the second polar body release in response to fertilization.
Keywords: Cortical granules, egg activation, T-type Cav3.2 channels.
Financing: FONDECYT 1221308
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Transcription factor SOX2 promotes chemoresistance in 3D in vitro model from prostate cancer cell lines.

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Background: Prostate cancer (PCa) is a highly prevalent tumor among men globally. Post-first-line treatment, patients may experience relapse presented as a castration-resistant phenotype. The development of metastasis and drug resistance is attributed to the high plasticity of cancer cells, reflected in the epithelial-to-mesenchymal transition (EMT). EMT induces both normal epithelial cells and cancer stem cells (CSCs), with canonical EMT transcription factors potentially linked to the upregulation of CSC markers. The transcription factor SOX2 plays a crucial role in malignant tumor development by promoting cell proliferation and survival and altering normal differentiation processes through interaction with other transcription factors such as KLF4 and TWIST. SOX2 expression could predict aggressive cancer behavior due to its dysregulation.
AIM: To evaluate overexpression of SOX2 and chemoresistance phenotype in 3D in vitro model from prostate cancer cell lines.
METHODS: In-vitro model was developed using primary culture cells lines obtained from prostate explants. Matrigel was used as matrix for 3D model developing. Relative protein and gene quantification were characterized by qPCR and western blot techniques. MTT viability assay was performed in this 3D in-vitro model with lentiviral SOX2 knock down (KD) with chemotherapeutics agents.
RESULTS: KD cell lines showed less capability for 3D structures formation. KD structures were ~80% smaller that control cell line structures. EMT transcriptions factors associated with SOX2, such as KLF4 and TWIST1, and CSC surface markers, CD44 and CD133, showed low expression according with SOX2 KD. Cell viability was significantly diminished in KD cell line in comparison with control cell line ($p < 0.05$, Mann-Whitney test).
CONCLUSIONS: This study indicates transcription factor SOX2 as a marker of chemoresistance in castration-resistant prostate cancer, correlating with diminished expression of EMT and CSC markers. These assays may validate the relationship between SOX2 and multi-drug resistance, plasticity leading to mesenchymal and CSC phenotypes.
Keywords: SOX2, prostate cancer, chemoresistance.
Financing: Fondecyt 1201704 and ANID, FONDAP 152220002
Acknowledgments: Ethical considerations: Approved ethical committee, Facultad de Medicina, Universidad de Chile n° 135-2015 y 083-2020.

Effects of estetrol on proliferation and viability in cell lines derived from estrogen-dependent pathologies

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Background. Estetrol is a weak estrogen with a high affinity for estrogen receptor alpha, which has shown anti-proliferative activity in breast cancer models, however, there has not been evaluation in other estrogen-dependent pathologies.
The objective is to evaluate estetrol in an *in vitro* cellular model of endometriosis and ovarian cancer.
Methods. The estrogen receptor positive endometriosis cell lines (Hs832 and 11Z), here used together with the ovarian cancer cell lines HEYA8 and SKOV3, also reported to express estrogen receptor. The breast cancer cell line MCF-7 was incorporated as a positive control for estrogen receptor alpha and proliferative response to estradiol. Cells were incubated with escalating concentrations of estradiol and estetrol for 24, 48 and 72 hours. Proliferation was evaluated by crystal violet staining, MTS assay and changes in cell cycle by flow cytometry. The presence or absence of estrogen receptors alpha or beta was determined by Western blotting. Results were determined statistically significant with p values of <0.05 by one way ANOVA.
Results. No alteration in viability or proliferation was observed in the presence of estetrol or estradiol (p=0,424 and p=0,225 at 48 hours) in 11Z endometriosis cell line. The Hs832 endometriosis cell line showed a modest but significant increase in viability with estradiol (10 nM) and a decrease with estetrol(1µM) (p=0,03 and p=0,01 at 48 hours respectively). The presence of the estrogen receptor was not detected in these cell lines. On the other hand, the presence estrogen receptor alpha was observed in the HEYA8 cell line together with a decrease in viability in response to estetrol (1µM,p=0,02 at 48 hours).
Conclusions. Estrogen Receptors were not detected in endometriosis cell lines in contradiction to previous reports. An effect of estetrol on estrogen-dependent pathologies only occurs at high concentrations. Current *in vitro* results do not promote further expansion to pharmaceutical applications for estretrol.
Keywords: Estrogens, endometriosis, cancer
Financing: FONDECYT #11170603 & #1220586, ANID-FONDEF #2110050, ANID–FONDAP #152220002 & #15130011, ICM-ANID, ICN2021_045

Spermatozoa separated by Percoll gradient in Salmo salar testicular macerate

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Background. Artificial reproduction is essential for the sustainable development of aquaculture. However, artificial reproduction of broodstock in captivity produces alterations in the processes of gametogenesis and spawning. Currently, spermatozoa are collected in fish farms by testicular dissection (testicular maceration). However, cellular variability in testicular maceration generates fluctuating fertilization rates. Our objective was to separate viable spermatozoa using the Percoll® gradient selection technique and evaluate its effect on sperm function.
Methods. Samples of testicular maceration from sexually mature males (*Salmo salar*), provided by Hendrix Genetics and diluted 1:1 (v/v) in Storfish® commercial solution, were divided into three groups: Control (C), T1 (30/90% Percoll®) and T2 (15/30% Percoll®). Flow cytometry was used to evaluate plasma membrane integrity (PMI), mitochondrial membrane potential (MMP) and ROS production level, and motility was analyzed using the CASA (Computer-Assisted Sperm Analysis) program.
Results: There were no differences between the groups for PMI ($P>0.05$). However, MMP and ROS levels were different between groups T1 and T2 compared to C ($P<0.05$). The curvilinear velocity (CV) was higher in group C compared to groups T1 and T2 ($P<0.05$), however, progressive motility was higher in T2 compared to T1 and C ($P<0.05$).
Conclusions: Percoll gradient centrifugation of testicular macerate allowed obtaining spermatozoa with greater progressive motility, high mitochondrial membrane potential, low levels of cytoplasmic ROS, which could increase artificial fertilization rates.
Ethical declaration: Approved by CEC from U. de La Frontera (Nº041_21 (12.05.2021)).
Keywords: Keywords. Artificial reproduction, testicular maceration, percoll gradient selection
Financing: Funding. ANID-FONDECYT POSTDOCTORADO 3210593
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Effects of in vitro fertilization temperature changes on embryonic development of atlantic salmon (salmo salar).

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Background. Embryogenesis in fish is linked to water temperature and has been described as a highly thermally sensitive stage. Fertilization in fish is defined as a subperiod of embryogenesis. Increasingly frequent and intense water temperature variations caused by climate change could occur during this process (fertilization) and affect embryonic development rates. There is little information on this subject. This study aimed to evaluate the effect of high *in vitro* fertilization temperatures in Atlantic salmon (*Salmo salar*) on embryonic development rates.
Methods. Gamete samples (oocytes and sperm) were exposed to different temperatures during *in vitro* fertilization (6 °C, 8 °C, 10 °C, 12 °C, 14 °C, 16 °C, 18 °C, and 20 °C). They were subsequently incubated in groups of 100 eggs (zygotes) at a constant temperature of 8 °C until hatching. The relationship between *in vitro* fertilization temperature and the development rate from fertilization to hatching of Atlantic salmon (*Salmo salar*) eggs was evaluated.
Results. The fertilization rate obtained at 24 hpf was 100% in all groups. At 120 UTA in the 14 -20 °C groups a low embryonic development rate was observed compared to the control group (8 °C) ($P<0.05$). From 180 - 300 UTA in the 18-20 °C groups a low embryonic development rate was observed compared to the control group ($P<0.05$). From 400 - 480 UTA no differences were observed in the embryonic development and hatching rates in all groups.
Conclusions. Our results indicate that increasing water temperature at the time of *in vitro* fertilization affects the embryonic development rates of Atlantic salmon, in stages of the period (relative age) according to the accumulated temperature units (UTA) that correspond to gastrulation (120 ATU) and somitogenesis (180 ATU).
Ethical declaration: Approved by CEC from U. de La Frontera (Nº041_21 (12.05.2021)).
Keywords: Embryonic development, in vitro fertilization, climate change.
Financing: Funding. ANID-FONDECYT POSTDOCTORADO 3210593
Acknowledgments: Hendrix Genetics S.A for providing biological material.

New enhancers born from old material: unravelling the origin of regulatory novelty in Xenopus

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Background: The origin of regulatory networks that control patterns of gene expression in a spatiotemporal fashion is a major questions in evolutionary developmental biology. To address this issue, we compared the frogs *Xenopus laevis* (*Xla*) and *Xenopus tropicalis* (*Xtr*), which diverged 48 million years ago, focusing specifically on new transcriptional enhancers involved in frontoparietal bone development.
Methods: The mineralization process of the frontoparietal bone was monitored by double histological labelling with Alizarin red and Calcein green. We examined gene expression using RNA-seq and regulatory landscape using ATAC-seq, during the differentiation of mesenchymal precursors into mature osteoblasts in both species.
Results: Histological labelling showed that the frontoparietal bone of *Xla* grows more slowly and in greater amounts than that of *Xtr.* When analysing the differences at the transcriptional level, we found that *Xla*osteoblasts exhibit an increase in the expression of genes associated with bone anabolism and the endoplasmic reticulum stress response. Finally, by observing the regulatory landscape of both species, we detected the emergence of new osteogenic enhancers in *Xla*, at the loci of the *ninj1, ccpg1*, *pnck* genes, all involved in cell survival.
Conclusions: We propose that in *Xla*, a larger skull bone requires a greater amount of protein synthesis, putting the cells under increased endoplasmic reticulum stress. To evade apoptosis caused by such a stress, the *ninj1, ccpg1*, *pnck* genes were recruited into the *Xla* osteoblastic regulatory network. We show that, for these genes, the accumulation of mutations in pre-existing sequences led to the emergence of new osteoblastic enhancers. These regulatory novelties contributed to a difference in the pattern of frontoparietal bone growth between these species.
Keywords: Osteogenesis, Enhancer evolution, Xenopus
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Cholesterol homeostasis during oocyte maturation: implication for the gamete´s functionality and viability

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Background: HDL receptor SR-B1-deficient females (SR-B1 KO) are infertile. Their oocytes accumulate unesterified cholesterol (UC) during maturation, resulting in high mortality and spontaneous activation rates. Aims: 1) To evaluate UC levels and expression of proteins regulating cholesterol metabolism in maturing oocytes and 2) To reduce UC levels in SR-B1 KO oocytes matured *in vitro* using Methyl-β-cyclodextrin (MβCD) and evaluate their functionality and viability.
Methods: Aim 1: C57BL/6 oocytes were retrieved at GV, GVBD, and MII (stages of maturation), fixed, stained with Filipin (UC staining), and observed using confocal microscopy. Fluorescence intensity was measured through ImageJ©. Proteomic analyses were performed using an open-access database (https://doi.org/10.1016/j.mcpro.2022.100481). Aim 2: Oocytes from SRB1 KO and WT females were matured *in vitro* in the presence/absence of MβCD. Oocyte activation was induced using $SrCl_2$. Statistics were performed by GraphPad 8.1.
Results: UC levels were higher in GVBD than in GV oocytes (p=<0.0001) and decreased in MII eggs (p=0.0009). The expression of proteins associated with synthesis (HGCSM1), esterification (ACAT), and uptake (SRB1) of cholesterol increased from GV to GVBD (p=0.0102, p=0.0085, and p=0.0002, respectively). From GVBD to MII, SRB1 was downregulated (p=<0.0001), while HGCSM1, ACAT, and APOA1 (efflux) increased their expression (p=<0.0001 and p= 0.0001, respectively). UC levels in SRB1 KO oocytes matured after MβCD incubation were reduced vs untreated KO oocytes (p=0.0491). After incubation with $SrCl_2$, 43% of KO oocytes activated and 38% died; the treatment of KO with MβCD maintained the activation rate (50%) but reduced oocyte demise to 6% (n=2 experiments).
Conclusion: The progressive reduction in intracellular UC levels during oocyte maturation is consistent with the increase in the expression of proteins involved in cholesterol esterification and efflux. Treatment of SR-B1 KO with MβCD during *in vitro* maturation allows the reduction of UC cholesterol levels and potentially improves their viability.
Keywords: Oocyte maturation, Unesterified cholesterol, Oocyte dysfunction, Female infertility
Financing: FUNDING: FONDECYT #1221376 and Technological Center of Excellence IMPACT #FB210024 (to D.B), and Fellowship FAMED y UANDES (to Y.O.)

Repetitive maternal restraint stress during pregnancy impairs fetal brain cortex development.

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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 neurodevelopment (i.e., neuronal/glial differentiation and maturation) are poorly understood. In this work, using a repetitive maternal restraint stress rat model, we assessed PS-induced morphological and molecular changes in the offspring developing brain, focusing on subpallial neuro/gliogenesis (i.e., the site where cortical GABAergic interneurons and some oligodendrocyte precursor cells (OPCs) are born).
Methods: Pregnant rats were split at embryonic day (E)0.5 in control and stress (repetitive restraint) groups. Rats from the restraint group were confined daily from E8.5 to a wired box for 2h/day. One subgroup of rats was euthanized at E17.5 (n=4/group), and embryos were recovered for brain extraction. Another subgroup was subjected to the stress protocol until parturition and pups were euthanized at postnatal day (P)9 (n=4/group) for brain extraction. Microscopic brain morphological analyses were performed in the subpallium and the cortex. In addition, neuronal differentiation was evaluated in the brain cortex by qRT-PCR, WB assays, and immunofluorescence, using cell specific markers.
Results: PS induces changes in the fetal subpallial neurogenic process at different levels, leading to a decrease in the number of interneurons in the brain cortex at E17.5 and P9. On the other hand, differentiation assays suggest an impairment of neuronal (GABAergic interneurons) and glial (OPCs) differentiation. Finally, the expression of specific GABAergic neuron markers such as somatostatin showed a significant decrease at P9, suggesting a PS-induced impairment in cortical interneuron development processes.
Conclusions: PS impairs fetal subpallial neuro/gliogenesis, altering neuron/glia differentiation processes and decreasing cortical interneuron populations at early postnatal stages.
Keywords: Neurogenesis, Interneurons, Subpallium, Fetal programming
Financing: Fondecyt 1211384 (LFB) – IMPACT Basal Funding 210024 – ANID Fellowship 21230442 (SO)

Menstrual fluid-derived extracellular vesicles as potential key mediators of endometriosis development

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Background: Endometriosis is a chronic gynecological condition affecting 10% of childbearing age women worldwide, greatly hampering their quality of life due to its varied symptomatology. Nonetheless, endometriosis etiopathogenesis is still unknown. Extracellular vesicles (EVs) are thought to be key mediators in disease progression, including in endometriosis. Menstrual Fluid (MF) is an underexplored tissue, and characterizing MF-derived EVs could shed light on the origins of endometriosis.
Methods: Endometriosis and healthy MF donors were recruited, and MF-plasma was obtained by Ficoll-Paque. MF-derived EVs were isolated by ultracentrifugation. EVs were characterized by Nanoparticle Tracking Analysis, flow cytometry, western blot, and transmission electron microscopy. EVs RNA content was determined by RNA-Seq, and their pro-angiogenic potential evaluated by angiogenesis assay with HUVEC cell line, and analyzed by ImageJ software. Results were normalized to vehicle control. Results are expressed as median±SEM. p-value<0.05 was considered significant.
Results: MF-derived EVs did not show significant differences in concentration (vs. $3.5x10^9$ vs. $5.7x10^9$ part/mL MF), size (169 vs. 168.5 nm), and morphology (p-value>0.05) between groups, but showed decreased CD63 tetraspanin enrichment in endometriosis [15.3 vs. 9.5 (fold-change); p-value<0.05, Mann-Whitney test]. Their RNA content showed enrichment in pro-angiogenic biological processes (adjusted p-value<0.05, GO enrichment analysis). When exposed to endometriosis-derived EVs, HUVEC cells showed a significant increase in the number of nodes (0.52 vs. 1.575), junctions (0.52 vs. 1.585), meshes (0.29 vs. 2.285), and segments [0.41 vs. 2.02 (fold-change)], compared to treatment with healthy EVs (p-value < 0.05, Mann-Whitney test).
Conclusions: MF-derived EVs from endometriosis and healthy women are distinct in their CD63 enrichment and RNA content, but show no difference in concentration, size, and morphology. As predicted by RNA-Seq, endometriosis MF-derived EVs have pro-angiogenic potential, compared to control EVs. These results point towards a pathophysiological role of MF-derived EVs in endometriosis establishment/progression.
Keywords: Endometriosis, extracellular vesicles, angiogenesis.
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Role of basigin on trophoblast migration and angiogenesis capacity during hypoxia

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Background: Basigin (BSG or CD147) is a highly glycosylated type-1 transmembrane protein involved in the induction of MMP synthesis, extracellular matrix lysis, tissue remodeling and angiogenesis. BSG is highly expressed on the surface of numerous types of malignant cancer cells, and it is induced by hypoxia-inducible factor (HIF1-α). Preeclampsia (PE) is characterized by a deficient trophoblast invasion of the decidua and an incomplete spiral artery remodeling. Thus, to determine whether BSG plays a role in the PE related processes in human pregnancy, it is crucial to study its expression in the first-trimester trophoblast cells.
Methods: BSG protein and mRNA expression was determined in HTR-8/SVneo first trimester human trophoblast cell line in response to 21, 5 and 1% oxygen for 24 and 48h of culture. Small interfering (si) RNA transfection was used to downregulate BSG expression. Transmigration and angiogenic capacity were assessed in HTR-8/SVneo-BSG-depleted cells using transwell and Matrigel-based tube formation assay, respectively. Results are expressed as mean±SD normalized to control. $P<0.05$ was considered significant.
Results: BSG protein expression was increased significantly after 24h at 1% oxygen (1.23±0.26 vs 0.96±0.06, p=0.01 Kruskal-Wallis test; n=4) compared to normal oxygen condition. HTR-8/SVneo-BSG-depleted cells showed a reduced transmigration capacity (0.63±0.12 vs 1±0.0, p= 0.03 Mann-Whitney test; n=4) but no changes in angiogenesis capacity after 24h of silencing ($p>0.05$ Mann-Whitney test; n=3) were detected.
Conclusions: These findings suggest that BSG has a role in trophoblast migration, a process that could be implicated in the pathogenesis of PE.
Ethical declaration: Approved by Ethical Ccientific Committee of Universidad de los Andes CEC2023039.
Keywords: Basigin, Trophoblast, migration
Financing: FONDECYT Postdoctoral #3230201, FONDECYT Regular #1241103

Domestication of DNA transposons into ubiquitous gene promoters in Xenopus frogs

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Background: Studies in mammals have revealed a key role for RNA transposons in the generation of novel regulatory elements. However, it is still unclear whether DNA transposons (specifically MITEs: "Miniature Inverted-repeat Transposable Elements") can be domesticated as ubiquitous promoters.
Methods: To address this issue, we performed a series of ATAC-Seq analyses on larval tissues of *Xenopus tropicalis* (*Xt*) and *Xenopus laevis* (*Xl*) frogs. MITE enrichment was assessed using RepeatMasker coordinates and, together with ATAC-Seq and RNA-Seq data, ubiquitous promoters harboring a MITE copy were identified.
Results: We found 26 promoters born from the Kolobok-N9 MITE subfamily, which is present in the genome of both *Xenopus* species that diverged 48 million years ago. Thus, these Kolobok transposon-derived MITEs were domesticated at ubiquitous promoters before the divergence between *Xt* and *Xl*. We detect convergent evolution of MITE domestication into ubiquitous promoters at three levels: (i) for orthologous genes, (ii) for neighboring genes located at the same locus, and (iii) for unrelated genes that nevertheless have important roles in the same cellular processes such as autophagy and lysosomal biology.
Conclusion: This work demonstrates that DNA transposons in the form of MITEs are highly versatile and can take other crucial cellular functions related to the expression of ubiquitous genes.
Keywords: Xenopus, Promoter, DNA-Transposons
Financing: FONDECYT 1190926. PhD fellowship ANID 21221189.

Embryonic Developmental Delay and Exencephaly Rates Associate to the Genetic Background in SR-B1 KO Mice

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Background. Mouse embryos lacking the HDL receptor SR-B1 (SR-B1 KO) show a high incidence of female-skewed exencephaly or cranial neural tube defect (NTD). NTD are fully prevented by feeding pregnant dams a vitamin E-enriched diet. We maintain two SR-B1 KO colonies: the original with a mixed C57:129 background (SR-B1) and a recently imported colony with an unknown proportion of B6 or 129 and higher NTD incidence (SR-B1/J). We hypothesized that the higher NTD incidence in KO embryos from the SR-B1/J colony was explained by a different genetic background. We also postulated that NTD was sex-dimorphic and vitamin E-preventable in both colonies.
Methods. Embryos from SR-B1+/- intercrosses were dissected on embryonic day 9.5. Neural tube closure and developmental progress were evaluated. Screening and sexing of embryos were achieved by PCR using extraembryonic tissues. Prevention of NTD by maternal vitamin E supplementation was evaluated in SR-B1/J embryos. The genetic background of females was determined using a SNP genotyping panel. Results are expressed as mean±SD or proportions. Statistics were performed using One-Way ANOVA, Student t-test or Fisher's test. $P<0.05$ were considered significant.
Results. SR-B/J embryos from all genotypes had fewer somites at E9.5 (15.4±5.8) compared to SR-B1 embryos (18.3±4.1; p=0.005). SR-B1/J-KO embryos had a higher NTD rate (57.1%) than SR-B1-KO embryos (18.5%; p=0.01). **I**n both colonies, near 66% of embryos with NTD were female. Maternal vitamin E supplementation reduced NTD incidence in SR-B1/J KO embryos from 57% to 17% ($p<0.05$). The proportion of C57:129 background was 50%:50% in SR-B1 females and 20%:80% in SR-B1/J females (p=0.05).
Conclusions. The developmental delay and increased NTD incidence in KO embryos in the SR-B1/J colony is associated with the higher 129 background in the former. NTD in SR-B1 KO embryos is female-biased and preventable by vitamin E intake, independently of the mouse background.
Keywords: Neural tube defects, Sexual dimorphism, Genetic background, SR-B1
Financing: FONDECYT #1221376 and ANID-Basal funding for Scientific and Technological Center of Excellence, IMPACT, #FB210024 (to DB).

Urolithins A and B prevent endometriotic lesion development affecting angiogenesis in mice

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Background: Endometriosis is a chronic disease characterized by the growth of endometrial tissue outside the uterine cavity. Current therapies are limited and associated with several side effects. Urolithins are natural compounds generated by the intestinal microbiota from ellagitannins and ellagic acid. We have demonstrated that UrolithinA (UA) and Urolithin B (UB) exhibit anti-proliferative, anti-migratory, anti-invasive, and pro-apoptotic effects, and downregulate the expression of angiogenesis-promoting genes in endometriosis in-vitro. Since angiogenesis is a key process involved in the establishment and progression of endometriotic lesions, we evaluated the effects of UA and UB on angiogenesis in endometriosis in-vivo.
Methods: Endometriosis was surgically induced in female BALB/c mice. Fifteen days post-surgery, the mice were treated with daily intraperitoneal injections of UA, UB, or PBS (Control, C). After 28 days, the animals were sacrificed, peritoneal fluid (PF) was collected, and endometriotic-like lesions were counted, measured, excised, and fixed. The vascularized area was assessed by CD31 immunohistochemistry, and the number of blood vessels was counted. The in vivo angiogenic potential of PF from UA-, UB-, and C-treated mice, or UA and UB alone, was evaluated using the quail chorioallantoic membrane (CAM) bioassay.
Results: UA completely prevented the development of endometriotic-like lesions ($p<0.001$), while UB significantly reduced implant size ($p<0.05$). UA directly assayed on CAM increased the number of blood vessel branch points ($p<0.05$), while UB had no effect. Additionally, PF from UA- and UB-treated mice had no effect on angiogenesis in the CAM assay ($p>0.05$). However, UB reduced the percentage of vascularized area and the number of blood vessels in mouse endometriotic-like lesions in-vivo ($p<0.05$).
Conclusions: UA and UB are effectivetreatments for endometriosis in our mouse model. Furthermore, UA completely prevented the development of the disease. However, further studies are needed to fully assess their anti-angiogenic potential.
Keywords: Endometriosis, Urolithins, Angiogenesis
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Characterization and determination of mRNA content of endometriosomes produced in vitro by bovine endometrial cells

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Background. The communication mediated by small extracellular vesicles (sEVs) has been considered to improve the production of in vitro embryos. Nevertheless, the molecular mechanism implicated in the modulation of the embryo development by endometriosomes are unknown. The objective of this investigation was to characterize and determine the mRNA content of endometriosomes produced by the endometrial cell lines CBR-BESC-UpEx and CBR-BEEC-UpEx.
Methods. Endometriosomes were isolated and purificated by Size-Exclusion Chromatography (SEC) and Ultrafiltration (UF). The morphology was analyzed by electronic microscopy (STEM), and specific biomarkers were identified by means of Western Blot. The size and concentration of sEVs was determined by Nanoparticle Tracking Analysis (NTA). The total RNA of endometriosomes was extracted and a RT-qPCR was performed to analyze the presence of mRNA of genes related to embryonic development.
Results. The rounded morphology observed by STEM and the immune detected specific biomarkers matches with the literature. The average diameter for the endometriosomes was 202.5 nm with a concentration of $2.39x10^8$ sEVs/mL and 214.9 nm with a concentration of $1.67x10^8$ sEVs/mL for CBR-BEEC-UpEx and CBR-BESC-UpEx respectively. It was found that in CBR-BEEC-UpEx had transcribed of OCT4, CDX2, IFNT2 and CAT genes, meanwhile CBR-BESC-UpEx had transcribed of OCT-4, DNMT1, IFNT2 and SOD1 genes.
Conclusions. The employed methodology allows to isolate and purify endometriosomes without harm their rounded morphology and preserving their biological activity. Besides, this investigation reports for the first time the characterization of endometriosomes biomarkers and their content of mRNA. In this aspect, transcribed fundamental genes related with the embryogenesis were identified, like OCT4, CDX2 or SOD1, revealing one molecular mechanism that modulates the embryonic development.
Keywords: Small Extracellular Vesicles (sEVs), mRNA, Embryo Development

Financing: This research was funded by the DI-UFRO Project DI20-0062, FONDECYT Project #1201166, and the National Doctoral Scholarship ANID #21191434, Chile.The project has been approved for implementation by the Scientific Ethics Committee (CEC) of the Universidad de La Frontera (N°125/20).

Metformin Impairs Pregnancy Outcomes in a Mouse Model of Maternal Hypercholesterolemia

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Background: Metformin has been suggested to improve maternal vascular dysfunction associated with pregnancy disorders; however, its use during pregnancy remains controversial. In this study, we evaluated the effects of metformin on maternal and fetoplacental outcomes in both healthy pregnant mice and a mouse model of high-cholesterol diet (HCD)-induced maternal vascular dysfunction during pregnancy.
Methods: Pregnant C57BL-6 mice were fed either a HCD or standard control diet (CD) between gestational day (GD) 13.5 and GD18.5. At GD13.5, one group of HCD and CD mice were treated with metformin (5 mg/ml) dissolved in water (4 groups in total, n=3). Mice were weight, and food and water intake were recorded. At GD18.5, females were euthanized, and placental and fetal outcomes were collected. Placentas were fixed in formalin for morphological analysis by HE staining. Results are expressed as mean±SD, and data were analyzed by two-way ANOVA followed by Sidak's post hoc testing. $P<0.05$ was considered statistically significant. Animal experiments were approved by CICUAL Facultad de Medicina, Universidad de Valparaíso (BEA 018-2024).
Results: HCD in late pregnancy reduced maternal weight gain compared to control mice (9±1 vs 3.7±2.1, $p<0.0001$), but metformin treatment decreased maternal weight either in HCD (-1±2.1) or control (8.3±2.1) groups ($p=0.0318$). No changes on water or food intake were found. The number of pups was reduced in HCD mice versus control groups (7±1.7 vs 9.7±0.6, $p=0.0304$). Metformin increased the number of reabsorptions in HCD mice, only (1.3±0.6 vs 0.3±0.6, $p=0.0339$). Fetal weight was reduced in HCD group versus control group (0.9±0.1 vs 1.2±0.07, $p<0.0001$); an effect that was exacerbated by metformin treatment. No difference in placental weight were found between groups, although fetoplacental ratio was reduced in HCD groups versus controls (9.2±1.3 vs 12.5±0.9, $p=0.0038$). Placentas from metformin-treated mice exhibited histological disorganization and an increased total vascular area, an effect that was more pronounced in those from females on a HCD.
Conclusions: Our preliminary data indicate that metformin negatively impact maternal weight during pregnancy. Female mice on a HCD treated with metformin exhibited significant illness. This study suggests that metformin has a detrimental effect on pregnancies complicated by pathophysiological hypercholesterolemia.
Keywords: pregnancy, high-cholesterol diet, metformin, fetoplacental outcomes
Financing: INICI UVA 22991, Universidad de Valparaíso.

Maternal circulating astrocyte-derived extracellular vesicles (ADEVs) can modulate prenatal stress-induced changes in fetal neurogenesis

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Introduction: Prenatal stress (PS) during pregnancy increases the risk of poor long-lasting neurodevelopmental outcomes in the offspring. However, the mechanisms by which PS alters fetal neurogenic processes and how stress signals are communicated from mother to fetus are poorly understood. Maternal brain astrocytes are reactive to stress conditions and astrocyte-derived EVs (ADEVs) can reach peripheral blood; thus, potentially acting as mother-to-fetus "stress signals". Using a rat model of prenatal stress based on repetitive restraint, we examined the effect of ADEVs on fetal neurodevelopmental processes. We focused on PS-induced morphological and molecular changes in fetal cortical neurogenesis and the role of ADEVs in modulating these alterations.
Material and Methods: Pregnant rats were divided into control and restraint (PS) groups starting at E0.5. From E7.5 to E14.5 or E16.5, the restraint group was confined 2 hours/day. Neural proliferation/differentiation assays were performed using BrdU injections at E14.5 and evaluated at E15.5 or E17.5. To analyze the role of ADEVs, four injections of EVs derived from control or corticosterone-treated astrocyte every 48hrs were performed and analyzed at E15.5. Neuronal differentiation and maturation in the fetal brain cortex were assessed by Western blot and immunofluorescence.
Results: PS reduced the number of intermediate progenitor cells at E15.5 and E17.5, suggesting premature neuronal differentiation. Expression of the maturation marker MAP2 increased in the restraint group at E15.5 but decreased at E17.5, indicating PS-induced impairment in neuronal maturation. ADEVs treatment showed a preventive effect, modifying the expression of neuronal differentiation markers in prenatally stressed animals.
Conclusions: PS induces significant alterations in fetal brains, characterized by accelerated neuronal differentiation, reduced neural progenitors at early stages, and a reduction of maturing neurons at later stages. ADEVs treatment provides a preventive effect, counteracting and/or modulating the effect of PS on fetal neurogenic processes.
Keywords: Exosomes, fetal programming, neuronal differentiation
Financing: Fondecyt 1211384 (LFB) – IMPACT Basal Funding 210024

NDRG1 gene editing by CRISPR-Cas9 to increase exosome production in endometrial cells cultured in vitro

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Background: *In vitro* fertilization (IVF) is one of the most efficient assisted reproduction techniques in cattle, but when compared to IVF in other species, its efficiency is low in terms of blastocyst formation rate. The use of small extracellular vesicles (sEVs), such as endometriosomes isolated from conditioned culture media, has been proposed to improve the efficiency of IVF. However, the isolation yield of sEVs from culture media is low. The aim of this work was to knockout the NDRG1 gene using CRISPR-Cas9 to increase the secretion rate of endometriosomes from CBR-BESC and CBR-BEEC endometrial cells.
Methods: CBR-BESC and CBR-BEEC cells were transfected with pCEB-RNAg- NDRG1 plasmid, which encodes for Cas9 endonuclease and a previously designed gRNA targeting the NDRG1 gene involved in exosome production regulation. Editing by the CRISPR-Cas9 system was confirmed by the T7 virus endonuclease I (EIT7) assay. Finally, the secretion rate of sEVs was determined by isolating and purifying them using size exclusion chromatography (SEC) and ultrafiltration, and their concentration was analyzed via nanoparticle tracking analysis (NTA).
Results: Correct transfection of pCEB-mRNAg-NRG1 and expression of Cas9 was confirmed by fluorescence microscopy visualization of the EGFP reporter gene, polystronically coexpressed with Cas9. The EIT7 assay confirmed NDRG1 gene knockout. Finally, NTA showed that CBR-BEECs with the edited NDRG1 gene significantly increased their sEVs secretion rate (65 sEVs/h), compared to their controls (13 sEVs/h).
Conclusions. The results obtained indicate that NDRG1 gene editing improves the secretion rate of sEVs from the CBR-BEEC cell line. From this perspective, the increased production of sEVs *in vitro* has a potential use for improving bovine IVF and other biomedical applications.
Keywords: Small extracellular vesicles (sEVs), CRISPR/Cas9, In vitro fertilization (IVF)
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Gestational COVID-19 changes the expression of BKCa channel subunits in human placenta

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Background. SARS-CoV-2 infection and COVID-19 pandemic increased the risk of preterm birth and obstetrical adverse outcomes. In gestational COVID-19 cases, placental vascular injury and thrombosis have been reported. The large-conductance calcium-activated potassium channels (BKCa) are highly relevant for placental vascular function, but there is no information about BKCa channels subunits expression in gestational COVID-19.
Aim. To determine the placental expression of BKCa subunits in gestational COVID-19.
Methods. RNA extraction (Total RNA mini kit, Geneaid) was performed from chorionic plate and chorionic villi of placenta from COVID-19 cases (n=16) and healthy controls (n=7). RT-PCR for BKCa α, β1, β2 and β3 subunits and 28S genes and agarose gel electrophoresis was made. Semiquantitative analysis was performed using Image J software. The BKCa subunit/28S relation was analyzed using the GraphPad Prism software and non-parametric U Mann-Whitney test was applied. Values are mean±SEM.
Results. In chorionic plate of cases, BKCa α subunit mRNA increased 3.9±0.4-fold, with especially marked increase in severe cases. There was a 3.4±1.4-fold increase of BKCa β1 subunit ($p<0.05$) and 1.8±1.0-fold increase of BKCa β3 subunit ($p<0.05$) in mild COVID-19 patients. There were no differences of BKCa β2 subunit expression in chorionic plate. In chorionic villi, there was a 0.4±0.6-fold decrease of BKCa β2 subunit mRNA ($p<0.05$) and 1.6±0.9-fold increase of BKCa β3 subunit mRNA ($p<0.05$), especially in mild COVID-19 patients. There were no differences of BKCa α and β1 subunits expression in chorionic villi.
Conclusions. The gestational COVID-19 seems to increase the expression of BKCa α, β1 and β3 subunits in chorionic plate and BKCa β3 subunit in chorionic villi. Also, COVID-19 seems to decrease the expression of BKCa β2 subunit in chorionic villi of placenta. This effect could be linked with a mechanism of compensatory vasodilation carried out by the placenta, in response to the vascular injury.
Keywords: BKCa, placenta, COVID-19
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Mitochondrial Transfer Improves mitochondrial dysfunction and oxidative-stress in Preeclamptic mesenchymal stem cells from menstrual fluid

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Background: Emerging research suggests that mitochondrial dysfunction plays a crucial role in the pathophysiology of PE, affecting not only the placenta but also the tissues of the gestational parent. A growing body of evidence indicates that defective decidualization and abnormal decidual responses contribute to PE, withendometrial-derived mesenchymal stem cells (eMSCs)playing a role in this process. Among the most recently discovered regenerative properties of MSCs is mitochondrial transfer (MitoT), which boosts the energetic profile of cells and influences proliferation, migration, metabolism, and cell fate decisions, all mechanisms that have been described in the pathophysiology of PE. Thus, we seek to demonstrate the relevance of mitochondria and its transfer in MSCs from menstrual fluid (MenSCs) and to evaluate the impairments of this process in patients with a history of PE.
Methods: Mitochondrial dysfunction and oxidative stress were evaluated in MenSCs from multiparous healthywomen (MenSC-Healthy) and in women with previous history of PE (MenSC-PE). MitoT between MenSCs was confirmed by flow cytometry and confocal microscopy. The occurrence and rescue of mitochondrial dysfunctionthrough MitoT in MenSC-PE was evaluated by mitochondrial membrane potential, ATP and ROS accumulation. A total of 3 different donors were used in three independent experiments. Statistical significance was assessed using non-parmetric un-paired Kruskal-Wallis test with Dunn multiple comparison test. *$p < 0.05$, **$p < 0.005$, ***$p < 0.001$.
Results. MenSC-PE mitochondria exhibit lower membrane potential (0.46x, $p=0.05$) and higher ROS accumulation (1.28x, $p=0.005$) compared to their healthycounterparts. We observed, for the first time, that menstrual fluid derived MSCs can both transfer and receive mitochondria to/from other MSCs, regardless of their healthy or disease status. Moreover, MitoT lead to a hyperpolarization of mitochondrial membrane potential compared to PE-derived MenSCs and reduced ROSlevels (0.8x, $p=0.05$), upon oxidative stress stimulation, tolevels comparable to MenSC-Healthy mitochondria.
Conclusions: The accumulation of ROS in MenSC-PE suggests a role in preeclampsia development by enhancing oxidative stress at the maternal-fetal interface. The capacity of menstrual fluid-derived MSCs for mitochondrial transfer and MitoT's ability to restore normal function highlight potential therapeutic avenues for mitigating oxidative stress and addressing preeclampsia-related complications
Keywords: Mitochondrial Transfer, Oxidative Stress, Preeclampsia

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Morphological and functional evaluation of güiña (Leopardus guigna) oocytes, in vitro maturation and parthenogenetic activation

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Background: The güiña is a vulnerable felid species. A female güiña was received in bad medical conditions and subsequently deceased in the CRFS-UdeC. The ovaries were collected during necropsy. The objective was to evaluate the morphology and functionality of güiña oocytes by in vitro maturation (IVM), and parthenogenetic activation (PA).
Methods: Cumulus oocyte-complexes (COCs) were subjected to IVM. Cumulus expansion and oocyte morphology were evaluated using the software ImageJ. PA was done using 7% ethanol for 5 minutes, and 5 µg/mL cytochalasin-B and 10 µg/mL cycloheximide for 5 hours. T-test was used to evaluate cumulus expansion and oocyte morphology (mean ± SD), and Wilcoxon test was used for embryo development ($p < 0,05$).
Results: 29 güiña COCs were collected,12 (41.4%) were grade II, 7 (24.1%) grade III, and 10 (34.5%) grade IV. Only grade II COCs were subjected to IVM. COCs showed a significant cumulus expansion after IVM (170.8 ± 33.9 µm vs. 222.7 ± 16.8 µm). 5/12 (41.6%) güiña oocytes were in Metaphase-II (MII). The zona pellucida (ZP) of güiña oocytes (19.8 ± 1.6 µm) was thinner than domestic cat ZP (21.9 ± 2.3 µm). No differences were found in the cytoplasm diameter between güiña (107.3 ± 6.5 µm) and cat oocytes (109.41 ± 3.7 µm), but the total diameter of güiña oocytes (149.1 ± 7.8 µm) was smaller than cat oocytes (164.9 ± 5.8 µm). Finally, MII güiña oocytes were activated, 100% cleavage at day-2, two morulae formed at day-5 (2/5, 40.0%), and one blastocyst at day-8 (1/5; 20.0%), this was similar to cat oocytes subjected to PA: cleavage (27/29; 93.1%), morula (19/27; 70.4%), blastocyst (6/27; 22.2%).
Conclusions: Güiña oocytes have a thinner ZP and a are smaller than domestic cat oocytes. However, their in vitro development was similar to domestic cat oocytes after PA.
Keywords: wild felids, oocyte maturation, embryo development
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Prenatal stress and neurodevelopment: effect of astrocyte-derived extracellular vesicles on neural stem/progenitor cells proliferation/differentiation balance

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Background. Prenatal stress (PS) increases the risk of poor neurodevelopmental outcomes in the offspring; though the mechanisms are not fully understood. Extracellular vesicles (EVs) may act as mediators of maternal-fetal stress communication, transferring cargo that alters the metabolism and physiology of recipient cells. In this context, it has been suggested that astrocyte-derived extracellular vesicles (ADEVs) can influence the biology of neurons and neural stem/progenitor cells (NSPCs). Additionally, ADEVs have been detected in the plasma of rats, and their cargo change under stress conditions. Nonetheless, their role in the regulation of PS-induced changes on fetal neurogenesis remains unknown. In this study, we assessed the effect of ADEVs on NSPCs proliferation and differentiation using an in vitro approach.
Methods. NSPCs were isolated from the pallium of rats at embryonic day (E)14.5, cultured as neurospheres, and divided into 5 experimental groups: (i) Control, no treatment; (ii) DMSO, (iii) Corticosterone (5 μM), (iv) ADEVs, and (v) ADEVs from astrocytes previously treated with corticosterone. Treated groups received 1 pulse of treatment every 24 hours (a total of 3 pulses). Cultures were analyzed by Western blot and immunofluorescence in adherent or suspension conditions for differentiation or proliferation assays, respectively.
Results. In proliferation conditions**,** ADEVs treated NSPCs showed an increase in the diameter and number of neurospheres, along with an increased expression of NSPC markers, such as Nestin and SOX2. In differentiation condition, ADEVs treatment appeared to stimulate cell differentiation through the expression of neuronal lineage markers.
Conclusions. ADEVs can influence/regulate the proliferation and differentiation of NSPCs, increasing their proliferative and differentiation capacity. This results highlight the potential role of ADEVs as mediators/modulators of mother-to-fetus communication under stress conditions.
Keywords: Neurospheres, Exosomes, Neuronal differentiation
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