XXVIII

REUNIÓN ANUAL
SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

LIBRO DE RESÚMENES

6-9 de Septiembre de 2017
Hippocampus Resort & Club
Concón, Chile

30 años

Compartiendo el conocimiento y la tecnología de la Reproducción y el Desarrollo

SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

Fundada del 30 de Abril de 1987

30 años

Compartiendo el conocimiento y la tecnología de la Reproducción y el Desarrollo

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SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

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PROGRAMA SCHRD 2017

Miércoles 6 de septiembre

14:00-15:30 **Inscripción de los participantes**

16:00-16:15 **Ceremonia Inaugural**
Palabras del presidente de la SCHRD
Alfonso Paredes, Universidad de Chile

16:15-17:00 **Conferencia Premio SCHRD 2016**
Coordina Alfonso Paredes
Camila Aguirre, Universidad de Chile
Assessment of complement system in response to early embryo signal in endometrium of assisted reproduction cycles

17:00-17:30 **Café**

17:30- 19:00 **Simposio I. De la genómica a la fisiología endometrial**
Coordina Alejandro Tapia

Ricardo Savaris, Universidade Federal do Rio Grande do Sul
Effect of randomized serum progesterone concentration on secretory endometrial histologic development and gene expression

María Cecilia Johnson, Universidad de Chile
Differential gen pattern in eutopic endometrium of infertile and fertile women diagnosed with endometriosis

Alberto Palomino, Universidad de Chile
The control of complement system activation in endometrium during the embryo receptivity phase

19:00-20:00 **Conferencia I**
Coordina Héctor Contreras, Universidad de Chile
Sergio Recabarren, Universidad de Concepción
El sueño de la reproducción y sus personajes

20:00 **Cóctel de Bienvenida**

Jueves 7 de septiembre

9:00-11:00 **Comunicaciones Libres I**
Coordina Mario Párraga, Universidad de Valparaíso

Carrasco-Wong I, Porras O, Casanello P
Oxidative stress-induced epigenetic transcriptional memory as a basis of programmed endothelial dysfunction in lga fetuses.

Patiño-García D, Cruz-Fernandes L and Moreno RD
The exposure to a phthalate and alkylphenol mixture alters the reproductive health of female mice through mir-200B-3P via the suppression of aromatase

Massa E, Lo Celso A, Zambrana A, Villarroel H, Madariaga MJ, Ghersevich S.
Administration of lactoferrin affects reproductive parameters in adult wistar rats

Figueroa E, Lee-Estevez M, Ulloa P, Valdebenito I, Farias J.G
Supplementation with α-tocopherol and ascorbic acid in the cryopreservation of atlantic salmon spermatozoa (salmo salar): effect on antioxidant enzymatic activity and sperm function.

Leonard B, Hamilton Thorne – GrupoBios
Técnicas de apoyo para la implementación de ICSI

11:00-11:30 **Café**

11:30-13:00 **Simposio II. Mecanismos Moleculares de la función espermática**
Coordina Emilce Díaz, Universidad de Antofagasta.

Claudia Tomes, Universidad de Cuyo
Membrane fusion during acrosomal exocytosis: molecules and something else.

Patricio Morales, Universidad de Antofagasta
Role of the ubiquitin-proteasome system (UPS) in the capacitation of human spermatozoa.

Alfredo Ramírez, Universidad Austral de Chile
Neuronal signaling in spermatozoa.

13:00-14:45 **Almuerzo**

15:00-16:00 **Conferencia II**
Coordina Gareth Owen, Pontificia Universidad Católica de Chile
Fernanda Parborell, IByME-CONICET
Oncofertilidad: nuevas estrategias en salud reproductiva y oncología.

16:00-16:30 **Café**

16:30-18:00 **Simposio III. Dinámica Folicular**
Coordina Alfonso Paredes, Universidad de Chile.

Marina Peluffo, CEDIE-CONICET–FEI–Hospital de Niños Ricardo Gutiérrez
Ruptura de la pared folicular y ovulación.

Marcelo Ratto, Universidad Austral de Chile
The study of follicular and luteal dynamics in domestic species and their application in the development of assisted reproductive technology or infertility problems

Hugo Ortega, Universidad Nacional del Litoral
Complex models to study ovarian alterations in farm animals

18:00-20:00 Sesión de Posters
Coordina: Gonzalo Cruz

Águila L, Treulen F, Arias ME, Felmer R.
Effect of synthetic metalloporphyrin (MnTBAP) supplementation on equine sperm function and zone pellucida-binding ability

Alanis C, Paredes A.
Recovery of the sympathetic innervation in rat ovary in period of fertility and subfertility.

Arroyo C, Sanhueza F, Fuentes F, Treulén F, Cabrera P, Arias M, Silva M, Felmer R.
Evaluation of htf medium as handling and capacitation medium for equine spermatozoa: preliminary results.

Carrasco A, Recabarren MP, Montalbán A, Gutiérrez M, Sandoval D, Sir-Petermann T, Recabarren SE.
Evaluation of the effect of chronic testosterone administration on insulin sensitivity (IS) in adult ooferectomized sheep exposed prenatally to an excess of testosterone (EPT).

Carrasco A, Díaz F, Gutiérrez M, Fuenzalida J, Montalbán A, Sandoval D, Recabarren MP, Sir-Petermann T, Recabarren SE.
Evaluation of the effect of prenatal exposure to testosterone (EPT) on morphometric parameters and plasma concentrations of hormones and glucose in female ovine fetus at 120 days of gestation.

Carrasco A, Recabarren MP, Montalbán A, Gutiérrez M, Sandoval D, Sir-Petermann T, Recabarren SE[1].
Testosterone administration decreases insulin sensitivity (IS) in adult female sheep born to pregnant testosterone treated mothers

Dumorné K, Valdebenito I, Risopatrón J, Figueroa E, Ulloa P, Cosson J, Lee Estevez M, Farías J.G.
Sperm of Pink Cusk-Eel (genypterus blacodes, schneider 1801): effect of PH, osmolality and temperature on sperm motility

Echiburú B, Maliqueo M, Pérez-Bravo F, Crisosto N, Flores C, Sandoval D, Recabarren SE y Sir-Petermann T.
Global DNA methylation since early infancy to adulthood in girls and boys born to women with polycystic ovary syndrome (PCOS).

Fuentes F, Sanhueza F, Arroyo C, Treulén F, Cabrera P, Arias M, Silva M, Felmer R.
Effect of the addition of dibutyryl-AMPc (DBAMPC), 3-isobutil-1-methylxanthine (IBMX) and methyl-β-cyclodextrin (MβCD) to the whitten´s medium on the quality of equine sperm

Gallardo LM, Patiño DF, Buñay J, Moreno RD.
Chronic exposure to a mixture of phtalates and alkylphenols modifies aromatase (*Cyp 19a1*), estrogen receptor *Erβ* and Pre-MicroRNAs modulating levels in spermatozoa, male germ cells affecting acrosome reaction (AR) and fertilization in mouse

Gutiérrez M, Sandoval D, Rojas D, Carrasco A, Díaz F, Recabarren MP, Recabarren SE.
Effect of an excess of prenatal exposure to testosterone (EPT) on the lung tissue structure and maturation in female ovine fetus at 120 days of gestation.

Henríquez S, Kohen P, Quilaqueo L, Godoy A, Orge F, Devoto L.
Estrogen metabolites in the regulation of angiogenesis in patients with polycystic ovarian syndrome**.**

Hernández A, Cuevas P, Vallejos C, Gallego I, Selman A, Vega M, Romero R.
Levels of microrna 23B in ovarian tissues and the effect of NGF in the levels of this microrna in epithelial ovarian cell lines

Jara B, Cheuquemán C, Sánchez R, Risopatrón J.
Addition of the antioxidant butylated hydroxytoluene in the freezing medium of cat spermatozoa: evaluation of effect on sperm motility and viability.

Lee-Estevez M, Figueroa E, Díaz R, Ulloa-Rodríguez P, Contreras P, Risopatron J, Valdebenito I, Cosson J, Farías J.
AMP-activated protein kinase (AMPK) activity is reduced in Spermatozoa of *salmo salar* after motility activation and under Cryopreservation conditions.

Molina P, Quiroz A, Rigotti A, Busso D.
Preliminary analyses of cell death pathways activated by excess cholesterol in mouse oocytes

Oróstica L, Ibáñez I, Tapia V, Hernández A, García V, Romero C, Vega M.
Activation of interleukin-6 (IL-6) pathway in endometria of women having polycystic ovarian syndrome (PCOS)

Párraga M, Smith D, Rejas C, Villena J, San Martín S, del Mazo J.
AntiMiRs against Mir-7013-5P, Mir-7116-5P and Mir-6373 generate differential effects on the translation of the reporter gene EGFP fused to *Rnf19A* or *Rnf19A-TPPG3* 3'UTRs in somatic versus germ cell lines.

Pérez G, López F, Ledezma R, Castellón EA, Contreras HR.
Distribution and expression of androgen receptor and 5-alpha Reductase II enzyme in human epididymis

Proietto S, Cortasa, SA, Corso MC, Inserra PIF, Leopardo, NP, Charif S, Schmidt AR, Di Georgio NP, Vitullo AD, Dorfman VB, Halperin J.
Luteal steroidogenesis and progesterone production in *Lagostomus maximus*: does prolactin play a central role by regulating the expression of the enzymes 3beta-and 20alpha-HSD?

Santander N, Lizama C, Quiroz A, Rigotti A, Busso D.
Identification of genes relevant for neural tube closure in SR-BI-deficient embryos using RNA-SEQ

Sepúlveda N, Bravo S, Sepúlveda C, Díaz R.
Seasonal variation in semen characteristics, ROS production and testosterone in rams

. **Serrano C**, Guzmán S, Cruz P, Torres CG.
Melatonin decreases proliferation and invasion of spheres derived from CF41.Mg canine mammary carcinoma cells.

Short SE, Bravo LA, Díaz R, Lee-Estevez M, Figueroa E, Zepeda AB, Farías JG.
Effect of barley dehydrin (P-80) on post-thawing sperm quality of atlantic salmon (Salmo salar).

Tapia A, Corrales P, Duarte D, Morales N, Pacheco V, Diaz ES.
Effect of vegetarian feeding on anthropometric parameters and sperm quality in the students of the University of Antofagasta.

Ulloa P, Contreras P, Figueroa E, Risopatrón J, Valdebenito I, Farías JG.
Characterization and short-term storage of the patagonian blenny (*eleginops maclovinus*) sperm.

Zepeda AB, Díaz R, Short SE, Valdebenito I, Farías JG, Moreno RD.
Reproductive parameters of atlantic salmon (*salmo salar*) from recirculation culture systems.

19:00- 20:00 Cheese and Wine

Viernes 8 de septiembre

9:00–11:00 Comunicaciones libres II
Coordina Héctor Contreras, Universidad de Chile

Mingo G, Valdivia A, Racordon D, Bravo ML, Sandoval A, González A, Retamal C, Cuello M, Nualart F, Sánchez B, Corvalán AH, Owen GI.
Vasculogenic mimicry: an alternative model of tumor irrigation in ovarian cancer

Cortez J, Nunda M, Bahamonde J, De los Reyes M, Palomino J, Torres CG, Peralta OA
Induction of bovine bone marrow mesenchymal stem cells into male germ cells using transforming growth factor-β superfamily growth factors.

Treulen F, Aguila L, Arias ME, Felmer R.
The synthetic metalloporphyrin MnTBAP protects equine sperm cell after cryopreservation.

Molina P, Quiroz A, Rigotti A, Busso D.
Ovarian reverse cholesterol transport: contribution of follicular fluid hdl and abca1 transporter to mouse oocyte cholesterol homeostasis.

Santander N, Rigotti A, Busso D.
Understanding cholesterol metabolism during early pregnancy

11:00-11:30 Café

11:30-13:00 **Simposio IV. Reproducción Animal**
Coordina Néstor Sepúlveda, Universidad la Frontera

Eduardo Aisen, Universidad del Comahue
Physico-chemical bases applied to spermatozoa cryoconservation

Rommy Díaz, Universidad de la Frontera
Study of the membrane lipid composition of Atlantic Salmon (*salmo salar*) spermatozoa and its relation with sperm functionality

Ricardo Moreno, Pontificia Universidad Católica de Chile
Cryopreservation and instrumental insemination in *Apis mellifera*

13:00-14:45 **Almuerzo**

15:00-16:00 **Conferencia III**
Coordina Carmen Romero, Hospital Clínico Universidad de Chile
Marta Tesone, IBYME-CONICET, Universidad de Buenos Aires
Role of Wnt/b-Catenin signal transduction pathway in rat follicular development and luteal function

16:00-16:30 **Café**

16:30-18:00 **Simposio V. Impacto de alteraciones nutricionales y endocrinas sobre el desarrollo intrauterino**
Coordina Gonzalo Cruz, Universidad de Valparaíso

Manuel Maliqueo, Universidad de Chile
Sex steroid and its relationship to normal and pathological pregnancies

Paola Casanello, Pontificia Universidad Católica de Chile
Early programing of obesity and metabolic disease in offspring of patients with pregestational obesity

Dolores Busso, Pontificia Universidad Católica de Chile
Is vitamin E a required nutrient during neurodevelopment?

18:00-19:00 **Conferencia de cierre**
Coordina Monika Greiner, Universidad Mayor
Andrés Gomberoff. Universidad Adolfo Ibáñez.
La física del divorcio: Modelos matemáticos que pueden resolver problemas de materiales magnéticos como arreglos de visitas de padres separados.

21:00-03:00 **Ceremonia de Clausura**
Entrega del Premio SCHRD 2017
Cena de Clausura

Sábado 9 de septiembre

10:00- 12:00 **Reunión de socios**

CONFERENCIA PREMIO SCHRD 2016

ASSESSMENT OF COMPLEMENT SYSTEM IN RESPONSE TO EARLY EMBRYO SIGNAL IN ENDOMETRIUM OF ASSISTED REPRODUCTION CYCLES

Aguirre C[1], Argandoña F[1], Kohen P[1], Sequeira K[1], Dittus S[1], Devoto L[1], Palomino A[1].
1Instituto de Investigaciones Materno Infantil, Departamento de Obstetricia y Ginecología Centro, Universidad de Chile.

Despite advances in assisted reproduction technology, embryo implantation continues to be a limitation of the process. The control of the activation of the complement system is fundamental for the survival of the embryo and the development of the pregnancy. The components of the complement system are expressed in the endometrium during the phase of endometrial receptivity. The objective of this work was evaluating the expression of C3 and complement regulator protein: deceleration factor (DAF: CD55) in response to human chorionic gonadotrophin hormone (hCG) in endometriosis of fertile women in spontaneous cycles and in infertile women in Cycles of assisted fertilization (IVF). Female endometrial biopsies were obtained in cycles of ovarian stimulation for IVF (n = 10) and fertile women during the mean secretory phase (n = 12). Endometrial explants were cultured with or without hCG for 24 hours and protein levels were quantified by Western blotting. In response to hCG, protein levels of C3 and DAF increased concomitantly in the endometrium of fertile women ($p < 0.05$) but did not change in IVF cycle endometriosis. The dysfunctional expression of the complement system in response to hCG in the IVF cycles, could generate an immunologically hostile environment compromising the embryonic survival and affecting its implantation.

FONDECYT 1140688.

CONFERENCIAS Y SIMPOSIOS

EL SUEÑO DE LA REPRODUCCIÓN Y SUS PERSONAJES

Recabarren SE.
Laboratorio de Fisiología y Endocrinología Animal. Facultad de Ciencias Veterinarias.
Universidad de Concepción, Chillán.

Hace 30 años hubo un sueño de muchos estudiosos de la ciencia de la Reproducción: Concebir la Sociedad Chilena de Reproducción y Desarrollo. De la fertilidad de sus personajes, se logró una fecundación exitosa cuyo fruto, después de una preñez de unos pocos meses, fue la Carta Constitutiva en forma de la Personería Jurídica otorgada por Decreto 1322 del 31 de diciembre de 1987 del Ministerio de Justicia y publicado en el Diario Oficial el 26 de enero de 1988.
Entre sus personajes, sobresale sin lugar a dudas el Dr. Eduardo Bustos Obregón, quien estuvo a cargo de la Sección correspondiente de la Sociedad de Biología y consiguió finalmente constituir la Sociedad Chilena de Reproducción afiliada a la Sociedad de Biología de Chile.
Es un honor rendir un homenaje a quien fuera el mayor impulsor y creador de la Sociedad en conjunto con los socios fundadores que aparecen en el documento que dio vida legal a la criatura. Las directivas que le siguieron, mantuvieron en alto, hasta el día de hoy los principios y objetivos de la Sociedad, y todos esperamos que siga siendo la luz que ilumine la creación del conocimiento y los fundamentos de la aplicación tecnológica para beneficio de la humanidad y sobre todo de nuestra sociedad chilena.
Fondecyt Nª 1140433.

EFFECT OF RANDOMIZED SERUM PROGESTERONE CONCENTRATION ON SECRETORY ENDOMETRIAL HISTOLOGIC DEVELOPMENT AND GENE EXPRESSION

Young SL[1], **Savaris RF[2]**, Lessey BA[3], Sharkey AM[4], Balthazar U[1], Zaino RJ[5], Sherwin RA[6], Fritz MA[1].
[1]Department of Obstetrics and Gynecology (CB#7570), 101 Manning Dr, University of North Carolina at Chapel Hill, NC, USA. [2] Departamento de Ginecologia e Obstetrícia, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2400, Porto Alegre, RS, 90035-003, Brazil.[3] Department of Obstetrics and Gynecology, Greenville Health System, 890 W. Faris Rd (Ste 470), Greenville, SC 29605, USA.[4] Department of Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK.[5] Department of Pathology, Hershey Medical Center, 500 University Drive, Hershey, PA 17033, USA.[6] Department of Obstetrics and Gynecology, The Whittington Hospital, National Health Service Trust, Magdala Ave, London N19 5 NF, UK

Progesterone (P) is required for normal embryo implantation and it is beneficial in assisted reproductive technology (ART) cycles; little is known about how endometrial function is expressed with different doses of P. This study investigates what doses of secretory phase P are associated with altered endometrial structure and function. In this case–control trial, 46 volunteers (age 19–34) underwent a single monitored natural cycle (10 controls) or modeled endometrial cycle after GnRH down-regulation. Modeled endometrial cycles were obtained by GnRH agonist down-regulation, transdermal estradiol (E2), and daily injections of P in oil for 10 days: 2.5mg (n=6), 5mg (n=6), 10mg (n=12) or 40mg (n=12), after the 10th day of E2. Endometrial biopsies were obtained on the 10th day of P exposure, or urinary LH surge. Analysis included histological dating, serum progesterone levels, microarray, RT-PCR, western blot and comparison with the GEO database.
A morphological delay appears in the 2.5 mg/day group; P ≥5 mg/day resulted in normal histology, but aberrant gene expression. Gene expression abnormalities occurred at higher and sub-physiological (P concentrations, without histological change. The expression of some endometrial receptivity-associated genes appeared multiphasic, suggesting sustained supraphysiological doses seen in ART treatment cycles may not be optimal.
Endometrial gene expression is differentially regulated by different doses of progesterone. The apparent multiphasic response of some genes to P dose suggests the possibility that P concentration kinetics may play a role in normal endometrial preparation for receptivity and histologic development is not a reliable measure of endometrial P action.
STUDY FUNDING/COMPETING INTEREST(S)
Supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 240239/2012-1 (RFS). Author have no competing interests.

DIFFERENTIAL GEN PATTERN IN EUTOPIC ENDOMETRIUM OF INFERTILE AND FERTILE WOMEN DIAGNOSED WITH ENDOMETRIOSIS

Johnson MC[1], Torres M[1], Fuentes A[1], González-Ramos R[1], Ribeiro C[2], Boric MA[1], Molina MC[2].
[1]Instituto de Investigaciones Materno Infantil (IDIMI), [2]Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Hospital Clínico San Borja Arriarán, Santiago, Chile.

Endometriosis is a proinflammatory invasive pathology with presence of endometrial tissue outside the uterus, and abnormal eutopic endometrial gene expression. Endometriosis affects 10% women at reproductive age, 50-70% infertile women, 10-50% with pelvic chronic pain and 4.9% asymptomatic fertile women. Peritoneal fluid is rich in several factors including NGF, a pleiotropic protein produced by neuronal/non-neuronal cells whose action is mediated by the receptors TrkA and p75NTR. Endometrium expressed all of them independently of the pain presence with high expression of both receptors during the secretory phase in eutopic endometria from endometriosis which did not significantly differ when women were classified in ovarian, peritoneal or deep-infiltrating endometriosis. NGF correlated positively with p75NTR in I/II and III/IV stages and with TrkA in III/IV stages of endometriosis (ASRM). NGF is related with IL-10, anti-inflammatory/immunosupressor cytokine, which was expressed in eutopic endometria increasing at late-secretory phase in fertile control, but not in endometriosis endometria. In endometriosis women classified as infertile (IE) and fertile (FE) women, NGF was higher than control, but TrkA in IE was lower than control and FE only at proliferative phase. Similarly, the strong IL-10 increase at mid/late secretory phases was not observed in IE, reduction concomitant with the maintained presence of progesterone receptor in secretory epithelial (PRA) and in stromal (PRB) endometria vs. control and FE. Conclusion: NGF system is overexpressed and may be involved in the progression of endometriosis, but not in the pain. A physiologic protector role of IL-10 during the implantation/invasion period, together with the abnormal presence of PR isoforms may contribute to infertility.
FONDECYT 1120074

THE CONTROL OF COMPLEMENT SYSTEM ACTIVATION IN ENDOMETRIUM DURING THE EMBRYO RECEPTIVITY PHASE

Palomino WA.
Instituto de Investigaciones Materno Infantil, Departamento de Obstetricia y Ginecología Centro, Universidad de Chile.

La adaptación de la respuesta inmune es fundamental para evitar el rechazo inmunológico del embrión. El sistema del complemento es parte importante de la respuesta inmune innata y el control de su activación es crucial para asegurar la supervivencia del embrión y el desarrollo del embarazo. Las proteínas reguladoras del complemento (CRPs) actúan a diferentes niveles de la cascada del complemento previniendo el daño tisular. En el modelo del ratón con anulación genética de la proteína reguladora del complemento cryy; funcionalmente homóloga al factor de desaceleración del complemento (DAF;CD55) en humanos, se produce pérdida embrionaria debido al depósito de productos de la activación del complemento C3. DAF;CD55 se localiza en el epitelio endometrial y su expresión es máxima en el periodo de receptividad embrionaria. DAF;CD55 se expresa en el epitelio endometrial bajo regulación paracrina, en respuesta a progesterona a través del factor de crecimiento epitelial (EGF) desde el estroma. DAF;CD55 incrementa su expresión en respuesta a la señal embrionaria la hormona gonadotropina coriónica humana (hCG) pero su incremento es anulado cuando se bloquea el receptor de progesterona (PR). La expresión disfuncional de DAF en el espacio embrión-endometrio puede producir la activación del complemento creando un ambiente inmunológicamente hostil comprometiendo la supervivencia del embrión. La regulación disfuncional de DAF;CD55 y la activación del complemento pueden explicar las fallas de implantación observadas en patologías como la endometriosis y la falla recurrente de implantación en los ciclos de reproducción asistida. FONDECYT 1140688

MEMBRANE FUSION DURING ACROSOMAL EXOCYTOSIS: MOLECULES AND SOMETHING ELSE

Tomes, C.
Instituto de Histologia y Embriologia de Mendoza (IHEM) "Dr. Mario H. Burgos". CONICET. Universidad Nacional de Cuyo. Mendoza – Argentina.

Secretory cells undergo regulated exocytosis to release biological compounds and to insert lipids and proteins into the plasma membrane. At the final stage of exocytosis, a fusion pore opens between the plasma and secretory vesicle membranes; typically, when the pore dilates the vesicle releases its cargo. My lab uses the exocytosis of the acrosomal vesicle of human sperm (the acrosome reaction or AR) as model system. Each sperm contains a single, very large and electron dense granule whose contents are secreted by a regulated exocytosis at fertilization. The acrosomal membrane fuses at multiple points with the plasma membrane that overlies the anterior part of the head. Joining of pores originates hybrid plasma membrane-outer acrosomal membrane vesicles. The AR is completed when vesicles and acrosomal contents are shed. My lab is interested in unveiling the molecular mechanisms that govern the AR. In the first part of my talk I will describe two proteins that connect the small GTPases Rab27 and Rab3. In the second part, I will introduce work we have conducted with Rab3A/Rab22A chimaeric proteins: the carboxy-terminus domain of Rab3A is necessary and sufficient to promote exocytosis whereas its amino-terminus prevents calcium-triggered secretion. We have applied funcional assays, electron and confocal microscopy and biochemical approaches to show that the Rab3A-22A chimaera blocks secretion because it stabilizes open fusion pores. In other words, vesiculation is not spontaneous as it was always assumed; rather, post-fusion regulation of the pores determines their expansion and the success of the AR. Sectyp 06/J484 y 06/J461, PICT-2013-1216 (C.N.T.)

ROLE OF THE UBIQUITIN-PROTEASOME SYSTEM (UPS) IN THE CAPACITATION OF HUMAN SPERMATOZOA

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Freshly ejaculated mammalian sperm cannot fertilize an egg unless they undergo a cascade of biochemical and physiological changes, collectively known as "capacitation". Protein kinase A (PKA) mediates sperm capacitation, although its regulation is not fully understood. In human sperm, PKA activity and cAMP levels rise at the beginning of capacitation; afterward, cAMP levels decrease but PKA activity remains higher than in non-capacitated spermatozoa. Phosphodiesterase activity remain constant during capacitation. The presence of the proteasome has been described in spermatozoa of many species. However, the importance of the UPS in sperm physiology has only recently been recognized. Here, we present evidence that the UPS is involved in human sperm capacitation and that interacts with the SACY/cAMP/PKA pathway.
Our results indicate that: 1) a number of proteasome subunits are phosphorylated by PKA during in vitro capacitation; 2) proteasome inhibitors block the enzymatic activity of the sperm proteasome and significantly reduce sperm capacitation; 3) proteasome inhibitors also block the phosphorylation of proteins that are PKA substrates (RRXpS/pT motif) that takes place during capacitation; 4) the level of ubiquitinated proteins increase during human sperm capacitation and this increase is further enhanced by proteasome inhibitors; 5) PKA inhibitors block the enzymatic activity of the sperm proteasome, while PKA activators significantly increase its activity; 6) finally, once the proteasome become activated by PKA, the proteasome modulates the activity of PKA. In conclusion, our data reveal feedback regulation between PKA and the proteasome during sperm capacitation.
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NEURONAL SIGNALING IN SPERMATOZOA

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Unlike postsynaptic neurons, whose activation is mediated by neurotransmitter and postsynaptic receptors interaction, the oocyte activation is mediated by cytosolic translocation of specific sperm phospholipase (PLCζ). At a subcellular level, and previous to gametic interaction, the events controlling sperm capacitation process are activated by chemical signals that trigger ion fluxes, sterol oxidation, synthesis of cAMP, PKA activation, tyrosine phosphorylations and calcium signaling, which correspond to second messengers also involved in exocytosis and growth cone guidance in neurons. Classically, sperm function associated with neural signals have been analyzed as a unidimensional approach (single ligand-receptor effect). For example, GABAergic, dopaminergic, serotoninergic, adrenergic, purinergic, cholinergic, and melatoninergic signaling have been reported. However, sperm cells are exposed to multidimensional signaling context, which understanding and global interpretation is a major challenge for basic and applied spermatology. Knowing that monoamine neurotransmitters regulate major functions associated with motor control, autonomic function, stress, emotion, sexual behavior and copula; and considering that mammalian sperm have functional monoamines receptors (DR2R, α2A and 5-HT) and monoamines transporters (DAT, NET, and SERT), we will show a biphasic model involved in motility, capacitation and sperm acrosome reaction. In this model, the excess of monoamines (DA, NE, and 5-HT) converging intracellularly in cytosolic degradation by monoamine oxidase (MAO), and whose derivatives including quinones and ROS, could regulate in vivo sperm functionality.

PRESERVING REPRODUCTIVE HEALTH AFTER CANCER: AN URGENT NEED IN LATIN AMERICA

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Oncofertility is an emerging interdisciplinary field that involves the study and development of new preventive and protective measures to reduce the impact of cancer treatments on reproductive health. Due to the increased survivorship of children and reproductive age patients treated with cancer thanks to the advances in anti-tumoral therapies, it is essential to understand its effects on future life quality and seek new techniques focused on preserving fertility. In patients older than 20 years who have received anti-tumoral treatments the rate of amenorrhea is 80%, the rate of premature ovarian failure (POF) is 90% and only 5-10% of these patients achieve spontaneous pregnancies. POF is characterized by the disappearance or dysfunction of the ovarian follicles in women under 40 years. POF can be consequence of treatment with chemotherapeutic drugs and/or radiation. Treatments for POF consist mainly of hormone replacement therapy but is not fully effective. There are various options to preserve fertility (GnRH agonists, cryopreservation of oocytes, embryos and ovarian tissue). In our laboratory, we propose two new strategies to protect the ovary in patients who are diagnosed with cancer and undergoing chemotherapy treatment: 1) Local administration of ceramide-1-phosphate sphingolipid (C1P) and 2) Local application of low intensity laser (LBI). We have observed that both C1P and LBI are able to preserve ovarian reserve in a POF model induced by cyclophosphamide in mice. Finally, given the increased survival of cancer patients of reproductive age, it is necessary to develop effective, safe and inexpensive strategies to protect the ovary and preserve fertility.
This study was supported by the ANPCYT (PICT 2015) and Roemmers Foundation

RUPTURE OF THE FOLLICLE WALL AND OVULATION

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Ovulation is a complex, inflammation-like process whereby a fully-developed follicle ruptures in response to the actions of the mid-cycle gonadotropin surge, releasing the cumulus-oocyte complex for passage into the reproductive tract and possible fertilization. Shortly before ovulation, the luteinizing hormone surge induces processes critical for fertility, including cumulus-oocyte expansion (C-OE), resumption of oocyte meiosis and follicle rupture. Recent data from nonprimate species indicate that these events involve a complex interaction of oocyte-, granulosa/cumulus-, and serum-derived factors. While some of the critical paracrine-acting factors have been identified, the molecular mechanisms responsible for initiating such complex processes are not fully understood. The release of the oocyte from the follicle requires highly coordinated events that include the degradation of type IV collagen, laminin, and fibronectin, which form the basement membrane surrounding the follicle, as well as the degradation of type I collagen within the follicle wall at the site of rupture. The degradation and cellular reorganization that takes place also allows for the invasion of newly forming blood vessels into the mural granulosa layer of the follicle. Such a high degree of extracellular matrix remodeling and cellular reorganization is associated with increased gelatinase, collagenase, and serine protease activities. The molecular mechanisms responsible for these critical processes within the preovulatory follicle are not fully understood. Thus, understanding the molecular and cellular processes involved in oocyte maturation, C-OE as well as follicle rupture would aid in the diagnosis or treatment of infertility and may also identify novel targets for a non-hormonal form of contraception.

THE STUDY OF FOLLICULAR AND LUTEAL DYNAMICS IN DOMESTIC SPECIES AND THEIR APPLICATION IN THE DEVELOPMENT OF ASSISTED REPRODUCTIVE TECHNOLOGY OR INFERTILITY PROBLEMS

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The application of ultrasonography in domestic animals has had a great impact in the understanding of the reproductive physiology in several species. The development of the ultrasonic image in real time, called B-mode, has facilitated a non-invasive serial visualization of structural changes with minimal alteration of the physiology resulting in a dynamic interpretation between structure and function.
The incorporation of the reproductive ultrasonography in ruminants during the 80's resulted in a significant comprehension of the ovarian function in cattle, stating for the first time that antral follicles growth in a wave-like pattern, something that had been postulated by Rajakosky in the 60's by anatomic and histologic studies. Although reproductive ultrasonography was used in several species, it was not until 2003 that appeared the first studied confirming the wave-like pattern of follicular development in human. The concept of follicular and luteal dynamics incorporated with the use of ultrasonography has allowed a better understanding of female cyclicity in domestic and non-domestic species. The bovine model has been the most studied and it was used as a template for elucidating physiological mechanism of ovarian function in different species. The use of the ultrasonography to study the reproductive process not only has improved the reproductive management in several species including bovine, sheep, horses and camelids, but also it has facilitated the efficiently incorporation of other reproductive technologies such as Artificial Insemination, Embryo Transfer and In vitro fertilization/embryo production. The main goal of this presentation is to describe the phenomenon of follicular wave in domestic animals, the methodology used to analyze and interpret the information recording by ultrasonography and to

give some examples of its application and uses in combination with some assisted reproductive technologies in animal science. We expect that that part of this information could be extrapolated for human reproduction. Proyecto Fondecyt Regular 1160934.

COMPLEX MODELS TO STUDY OVARIAN ALTERATIONS IN FARM ANIMALS

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Cystic ovarian disease (COD) is a major factor contributing to poor reproductive efficiency of lactating dairy cows. However, the pathogenesis of COD has not been clearly established. Cysts develop from preovulatory follicles that fail to ovulate, persist and then interfere with normal ovarian function. The most widely accepted hypothesis is that COD is the result of a 'hormonal-imbalance' within the hypothalamic-pituitary-gonadal axis. The study of the processes that lead to ovulatory failure and persistence of the dominant follicle is the key to understanding the pathogenesis of COD. The major difficulty in investigating cysts is that its formation can only be retrospectively recognized, after the follicle has undergone extensive pathological changes. Therefore, prediction of the time of cystic structure formation through follicular development in experimental models is a formidable opportunity to understand their pathogenesis. In this sense, numerous experimental models have been developed to induce the formation of follicular cysts. However, the possible role of intermediate levels of progesterone in promoting the formation of ovarian follicular cysts has been investigated only in short-term models without reaching the time of follicular persistence necessary to define ovarian structures as follicular cysts. Thus, we analyze the endocrine profile, growth dynamics and histological characteristics of persistent ovarian follicles/cysts developing in response to long-term administration of intermediate levels of progesterone. Persistent follicles induced by this model appear to mimic cystic follicles that arise spontaneously, providing a new tool to study their early stages. The endocrine profile, growth dynamics and histological characteristics of persistent ovarian follicles are analogous to those of spontaneous cysts.

PHYSICO-CHEMICAL BASES APPLIED TO SPERMATOZOA CRYOCONSERVATION

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Preservation of cell life at temperatures well below 0 ° C, where a physical change from liquid state to solid state occurs, is the goal of cryopreservation.
Cellular stress factors (membrane alterations, ionic imbalances, etc.) caused by apoptosis, hypothermia or freezing are present, for the most part, in all three processes.
The understanding of the basic physicochemical processes involved in cryopreservation makes it possible to define new semen processing techniques with a higher rate of morphological and functional integrity of frozen-thawed spermatozoa. Concepts and principles such as latent heat, melting point, ice crystal formation, metastable equilibrium, cryoscopic decrease, osmotic pressure, osmolarity and tonicity of solutions, among others, are the basis of dilution, cooling, freezing and thawing of seminal doses.
New tools such as the use of antifreeze proteins, seminal plasma, vitrification, lyophilization and preservation above 0 ° C are applicable by virtue of knowledge of the basic principles of cell cooling and freezing.

STUDY OF THE MEMBRANE LIPID COMPOSITION OF ATLANTIC SALMON (*Salmo salar*) SPERMATOZOA AND ITS RELATION WITH SPERM FUNCTIONALITY

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Atlantic Salmon (*Salmo salar*) is one of the best known and widely commercialized salmonids in the world, and has great productive potential in our country. Cryopreservation has been used for reproductive practices, conservation of germplasm and improvement of genetic resources. This technique offers several benefits for aquaculture and conservation biology, including the availability of gametes out of spawning season, allowing protection of strains with biotechnological interest and preservation of genetic profile of endangered species. However, its use has been limited due to poor post-thawing sperm quality. The composition and integrity of plasma membrane play an important role in fertilization capacity of spermatozoa, the spermatozoa-oocyte interaction and sperm cryotolerance. For this reason, biochemical composition of the membrane is one of the main areas of interest in the study of sperm physiology and pathology. In fish, there is little information about the lipid composition and structure of the membrane and the important role it plays in the sperm-oocyte interaction. Therefore, the aim of this project is to study the lipid composition of the sperm plasma membrane and its influence on the quality and functionality of Atlantic salmon spermatozoa in fresh and cryopreserved conditions. This will increase our knowledge about sperm biology and biochemistry and could help us to improve the process of cryopreservation and the application of this biotechnological tool in the reproduction of fish in aquaculture industry.
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CRYOPRESERVATION AND INSTRUMENTAL INSEMINATION IN *Apis mellifera*

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In honey bees, the queen is fertilized only once in her life when she is 20-25 days old, and this enables her to produce about 2000 larvae daily throughout her life. In a colony, the workers are infertile diploid females, whereas males (drones) are haploids, as a product of oocyte's parthenogenetic activation. In the apiculture industry, the production of queens is controlled by the caloric intake of females, and assisted reproduction by instrumental insemination of spermatozoa in the uterus of a virgin queen. However, males can be obtained only when there is pollen (spring-summer). That is, female queens are inseminated in a particular period of the year, only because the availability of semen (drones). This biotechnological problem in the industry could be solved by the development of the semen cryopreservation technique, in which this material could potentially be available at any time. The aim of this work was to develop a methodology of freezing for semen drones in order to use it in the instrumental insemination of virgin queen bees. By evaluating different freezing protocols, we managed to reach one in which the post-freeze sperm survival was more than 80%. After instrumental insemination, we were able to produce workers in queens inseminated with frozen / thawed semen. Therefore, it is possible to use frozen / thawed drone semen in the production of workers from inseminated queens instrumentally.

ROLE OF WNT/B-CATENIN SIGNAL TRANSDUCTION PATHWAY IN RAT FOLLICULAR DEVELOPMENT AND LUTEAL FUNCTION

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The aim of this study was determining the involvement of Wnt/β-Catenin pathway in ovarian function of superovulated rats. Immature rats were subcutaneously injected with eCG followed by hCG 48 hours later. The day of hCG administration, Wnt/β-Catenin inhibitor (XAV939 group) or vehicle (DMSO, control group) was administered into the bursa of both ovaries. Animals were euthanized 24 or 48 hours after XAV939 or vehicle administration. Follicle and Corpus Luteum (CL) development were analyzed in ovarian sections from each group and serum progesterone (P) concentration was measured. In CL protein extracts were determined the content of apoptotic and steroidogenic regulators proteins. In addition, the proliferation marker PCNA, and AKT/ ERK signaling pathways were analyzed. Finally, luteal VEGF abundance and vascular development through lectin staining were determined.
We found that inhibition of Wnt signaling impaired CL development, with a decrease in the number of CL balanced by a high number of cysts (unruptured follicles containing an oocyte); decreased circulating progesterone levels, likely due to a decrease in CL StAR content.
XAV939 administration increased pro: anti-apoptotic protein ratio, which enhanced apoptosis of the CL cells. In addition, the percentage of endothelial cell area in the CLs and the abundance of luteal VEGF were diminished after administration of XAV939. Wnt/β-Catenin inhibition decreased ERK phosphorylation and luteal PCNA protein levels. PCNA immunohistochemistry revealed a decrease in the staining of this protein in endothelial cells and small luteal cells.
These results describe for the first time that Wnt/β-catenin system regulates ovulation and CL function in gonadotropin–treated rats.
This study was supported by CONICET, ANPCYT, UBACYT, Rene Baron and Williams Foundation.

EARLY PROGRAMING OF OBESITY AND METABOLIC DISEASE IN OFFSPRING OF PATIENTS WITH PREGESTATIONAL OBESITY

Muñoz E[3], Jaramillo A[3], Carrasco-Wong I,[1,4] Hernández C[1,2], Soto G[1,2], Garmendia ML[3], Krause BJ[2] Uauy R[2,3], Castro-Rodriguez JA[2], **Casanello P[1,2].**
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Near 60% of woman in reproductive age in Chile are overweight or obese with detrimental consequences for their offspring´s health at long term. Maternal obesity previous and during pregnancy impinge an increased cardiometabolic and obesity risk in the progeny. In this context inflammation seems to play a central role in the development of pregnancy complications associated to obesity as well as the origin of obesity in the offspring. Increased levels of pro-inflammatory mediators and markers are reported in maternal and fetal plasma as well as placental tissue from pregnancies affected by maternal obesity. Notably increased inflammatory markers have been found in toddlers, but if these markers are present at the moment of birth is completely unknown.
During this talk a review of the literature and results from our research team will show systemic inflammatory and metabolic markers, expression of pro and anti-inflammatory genes in immune cells from umbilical cord and epigenetic mechanisms in endothelium from offspring born to women with pregestational normal weight and obesity.
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SEX STEROID AND ITS RELATIONSHIP TO NORMAL AND PATHOLOGICAL PREGNANCIES

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During pregnancy, sex steroids participate in the regulation of maternal metabolism and placental function. Therefore, variations of maternal sex steroids concentrations could compromise the normal course of pregnancy affecting the maternal health and fetal development. In this regard, progesterone and estrogens modulate the synthesis and release of angiogenic factors by placental cells, which regulates trophoblastic invasion and uterine artery remodeling. In contrast, androgens reduce the uterine-placental blood flow and placental amino-acid transport affecting the fetal growth. Placenta is main source of sex steroid during gestation being P450 cholesterol side chain cleavage (P450scc) and P450 aromatase, the rate-limiting enzymes in the biosynthesis of progesterone from cholesterol and estrogen from androgens, respectively. Interestingly, pregnant women with pregestational type 2 diabetes and polycystic ovary syndrome (PCOS) and those with gestational diabetes mellitus (GDM) and preeclampsia exhibit altered sex steroid concentrations along with changes in the expression and/or activity of placental P450scc and P450 aromatase. Moreover, recently, we have observed that obese pregnant women without pregnancy complications exhibit lower progesterone and higher testosterone levels during the first and third trimesters of gestation compared to normal-weight. Moreover, alterations in the placental protein expression of enzymes that synthesize progesterone and estrogen were observed in these women. Therefore, all these data together indicate that maternal variations in sex steroid concentrations during pregnancy could be a consequence of an altered maternal milieu contributing with derangements associated with pregnancy pathologies. FONDECYT 11130250

IS VITAMIN E A REQUIRED NUTRIENT DURING NEURODEVELOPMENT?

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Organogenesis starts with the formation of the neural tube, which later gives rise to the nervous system. Neural tube closure takes place during early gestation, between embryonic days 8.5 (E8.5) and E9.5 in mice. At that stage, before the formation of a functional placenta (E12), embryonic nutrition is achieved by the yolk sac, extraembryonic membrane that expresses several lipoprotein receptors involved in lipid transport. One of these receptors is Scavenger Receptor Class B Type I (SR-BI), involved in the bidirectional transport of cholesterol and vitamin E to/from HDL. We showed that approximately 50% of SR-BI$^{-/-}$ embryos fail to close the anterior neural tube and develop exencephaly, a perinatal lethal condition. Compared to wild-type embryos, SR-BI$^{-/-}$ embryos have very low vitamin E content and high levels of reactive oxygen species (ROS). SR-BI$^{-/-}$ embryos with neural tube closure defect (NTD) have reduced expression of genes previously shown to be required for neural tube formation, such as Pax3, Alx1 and Alx3. Interestingly, maternal α-tocopherol dietary supplementation can prevent NTD almost completely (from 54% to 2%, $p<0.001$) in SR-BI$^{-/-}$ embryos and normalize ROS levels and gene expression. In humans, the incidence of NTD has been reduced in 50% by preconceptional folic acid supplementation. However, this malformation has not completely been abolished (current prevalence: ~5/10,000 cases), supporting the need for the implementation of additional NTD-preventive strategies. Ongoing research in our lab is aimed at studying E metabolism during pregnancy and the potential contribution of vitamin E deficiency – by abnormal maternal status and/or transport to embryo - in the ethiopathogenesis of NTD. Funding: FONDECYT 1141236 (to D.B.), PhD fellowship CONICYT 21130444 and School of Medicine PMD-04/16 (to N.S.).

LA FÍSICA DEL DIVORCIO: MODELOS MATEMÁTICOS QUE PUEDEN RESOLVER PROBLEMAS DE MATERIALES MAGNÉTICOS COMO ARREGLOS DE VISITAS DE PADRES SEPARADOS.

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Los individuos divorciados que tuvieron hijos con sus ex parejas y que ahora tienen una nueva suelen tener problemas logísticos que son fuente de gran estrés. ¿Es posible conseguir un regimen de visitas que permita a todos los niños de la nueva familia estar juntos fin de semana por medio? Así además, la nueva pareja gozará de un romántico fin de semana sin niños el resto de los fines de semana. La posibilidad de un arreglo así se dificulta en la medida que los miembros de la actual familia tienen más hijos con más ex parejas, ya que cualquier decisión que se tome respecto del régimen de visitas afecta a una vasta red de personas. La pregunta social se transforma en una científica: asumiendo buena voluntad de todos los individuos, ¿es posible encontrar un régimen que deje a todos los miembros de esta red felices ? De no ser así, ¿es al menos posible minimizar el número de individuos infelices?. Solo la respuesta a la segunda pregunta es positiva. Curiosamente, la solución es matemáticamente equivalente al problema de encontrar el mínimo de energía de ciertos materiales magnéticos conocidos como vidrios de espín. Es así como podemos aprender física pensando en problemas sociales. Y viceversa.

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OXIDATIVE STRESS-INDUCED EPIGENETIC TRANSCRIPTIONAL MEMORY AS A BASIS OF PROGRAMMED ENDOTHELIAL DYSFUNCTION IN LGA FETUSES

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Large-for-Gestational-Age fetuses (LGA) have increased oxidative-stress (OxS) markers in cord-blood, revealing a detrimental milieu during development. However, what cellular/molecular components of the antioxidant-machinery play a role in this altered response is missing. We proposed that human-umbilical-artery-endothelial-cell (HUAEC) from LGA have an altered antioxidant-machinery and Epigenetic-Transcriptional-Memory (ETM) programmed by OxS.
To evaluate cellular antioxidant-capacity, HUAEC-LGA were transduced with pHyper-probe and *in-vivo* exogenous H2O2-induced fluorescence measured. Antioxidant proteins (GPX1, HMOX1, NRF2, SOD1, NOX1) where quantified by Western-blot. Basal Transcript levels of these genes or using a double-OxS protocol were quantified by qPCR. Open chromatin state was evaluated by DNase-HS assay. ChIP for NRF2, RNAP2 and RNAP2-pS5 in the HMOX1 core promoter and Enhancers 1 & 2 performed.
Hyper-transduced HUAEC-LGA showed increased EC50 in a H2O2-response curve. No differences at the protein level of antioxidant genes were observed. Basal *NRF2* and *HMOX1* mRNA were down and upregulated, respectively. HUAEC-LGA show an open chromatin-structure in the *GPX1* gene and a H2O2-induced its over-expression. In HUAEC-C and EA.hy926 only *HMOX1* showed a ETM-like response when challenged with the double-OxS protocol. In H2O2-primed cells, DNase-HS assay revealed an open chromatin-structure at *HMOX1* gene core-promoter and Enhancer-2; associated to paused-RNAPII and NRF2 enrichment, respectively. These results evidence for the first time a H2O2-induced ETM in human endothelium by OxS.
Summarizing, HUAEC-LGA have a highly functional antioxidant-machinery, related to altered gene expression and ETM-like response. However, *in-vitro* OxS-primed response of *HMOX1* gene in human endothelium suggests that OxS requires additional factors to generate a ETM in antioxidant coding-genes.
Funded by FONDECYT #1171406, #1141195. ICW holds a CONICYT PhD fellowship

THE EXPOSURE TO A PHTHALATE AND ALKYLPHENOL MIXTURE ALTERS THE REPRODUCTIVE HEALTH OF FEMALE MICE THROUGH MIR-200B-3P VIA THE SUPPRESSION OF AROMATASE

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Humans are chronically exposed to endocrine-disrupting chemicals (EDCs) that affect the reproductive health, being, microRNAs (miRs) in reproductive tissues, emergent targets of EDCs. Here, we analysed changes in the ovary, miR biogenesis mechanism and their effects on the fertility of chronically exposed adult female mice to a cocktail (in drinking water) of five EDCs containing 0.3 mg/kg/d of each phthalate (DEHP, DBP, BBP) and 0.05 mg/kg/d of each alkylphenol (NP, OP), from conception to adulthood. The onset of puberty and estrous cycle were evaluated and in the ovary, preantral and antral follicles numbers were assessed by histology. Expression levels of genes involved in the steroidogenesis and miR biogenesis, as well as mature miRs were measured by RT-qPCR and western blotting. Plasma levels of progesterone, testosterone and estradiol and the fertility rate were also evaluated. A delay in the puberty and a disrupted the estrous cycle were found along with a decrease of estradiol and progesterone levels and the relative weight of ovaries. This mixture also altered the preantral to antral follicle progression, and reduced the number of corpora lutea. Finally, there was a decrease in mRNA and protein levels of STAR and CYP19A1 and an increase of miR-200b-3p levels along with an over-expression of *Drosha* were observed. As a result, the fertility rate and litter size decreased. In conclusion, our results suggest that a chronic exposure to the mixture is targeting the population of ovarian follicles inducing a reduction in steroidogenesis, which affects the female reproductive cycle and fertility through a mechanism dependent of miR-200b-3p.
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ADMINISTRATION OF LACTOFERRIN AFFECTS REPRODUCTIVE PARAMETERS IN ADULT WISTAR RATS

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We previously reported that lactoferrin (LF) is present in human oviduct secretion and binds to human gametes. Our results indicated that LF decreased gamete interaction *in vitro*. The aim of this study was to investigate LF effect on the *in vivo* reproductive process and its binding to spermatozoa in a rat model. Wistar female rats (10-11 weeks old, n=24) were randomly assigned to one of four treatment groups, receiving daily an i.p. injection either of: 100, 200 or 400 mg LF/kg or 0.9% sodium chloride (controls), during one complete estrous cycle. On the respective proestrous day, treated females were kept with a male rat. Number of pregnant females and born pups was registered. To study LF binding to rat spermatozoa, motile sperm were incubated with LF-fluorescein isothiocyanate under capacitating conditions. None of the animals treated with 100 mg LF/kg got pregnant. Statistical analysis revealed that 100mg LF/kg administration was associated with a reduction of the pregnancy rate compared to control ($p<0.05$). Administration of 200mg LF/kg reduced the mean number of pups (11.6 □ 0.5 vs. 7.5 □ 2.0; $p<0.01$). Fluorescence microscopy indicated that LF was able to bind to rat spermatozoa over the entire cell. These results suggest that LF administration could have negative dose dependent effects on *in vivo* rat reproductive process. Although the mechanisms involved in the *in vivo* effects of LF remain to be elucidated, they might result from a decreased gamete interaction in the presence of the protein as previously reported on human.

SUPPLEMENTATION WITH α-TOCOPHEROL AND ASCORBIC ACID IN THE CRYOPRESERVATION OF ATLANTIC SALMON SPERMATOZOA (Salmo salar): EFFECT ON ANTIOXIDANT ENZYMATIC ACTIVITY AND SPERM FUNCTION

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Supplementation of cryopreservation medium with α-tocoferol and ascorbic acid prevents lipid peroxidation and ROS-induced damage; however, these effects have not been studied in salmonids spermatozoa. The objective was to determine the protective effect of antioxidants on enzymatic activity and fertilizing capacity of cryopreserved spermatozoa of Atlantic salmon. Spermatozoa were frozen in Cortland® medium containing 1.3M DMSO, 0.3M glucose, 2% BSA and different antioxidants: G1: no antioxidants; G2: α-tocoferol (0,1mM); G3: α-tocoferol (0,5mM); G4: ascorbic acid (1mM); G5: ascorbic acid (10mM); G6: α-tocoferol/ ascorbic acid (0,1mM/1mM); G7: α-tocoferol/ascorbic acid (0,5mM/10mM); fresh semen was used as control. Straws (0.5mL) were frozen at -68°C/min (Freeze Control®) and thawed at 35°C (1x109 esp/mL). Lipid peroxidation, Glutathione (GSH/GSSG), Glutathione peroxidase (GPx) and Catalase (CAT), mitochondrial membrane potential (ΔΨM) and fertilization rate were evaluated post-thawing. Spermatozoa treated with α-tocoferol (0.1mM and 0.5mM) and α-tocoferol/ascorbic acid (0,1mM/1mM and 0,5mM/10mM) showed reduction of [MDA] (2,10±0,6nmol/ml, 2,21±0,7nmol/ml, 1,99±0,4nmol/ml y 2,10±0,5nmol/ml, respectively) compared to G1 (3,87±0,84nmol/ml; $p<0,05$). Treatment with α-tocoferol/ascorbic acid (0,1mM/1mM and 0,5mM/10mM) exhibited increased GPx activity (21,90±3,2nmol/min/ml and 20,90±2,6nmol/min/ml) and GSH/GSSG (0,99±0,2μM y 0,97±0,2 μM) compared to G1 (7,9±1,1nmol/min/ml; 0,51±0,1μM respectively, $p<0,05$); while Catalase displayed increased activity (1,20±0,2 U/ml) in spermatozoa treated with α-tocoferol/ascorbic acid (0,1mM/1mM) respect to G1 (0,79±0,1U/ml, $p<0,05$). Additionally, ΔΨM was significantly higher (68,5±3,9% and 67,1±5,1% respectively) in spermatozoa frozen with α-tocoferol/ascorbic acid (0,1mM/1mM and 0,5mM/10mM) compared to G1 (50,5±6,7%, $p<0,05$); while fertilization rate was increased (93,5±6,3%) by treatment with α-tocoferol/ascorbic acid (0,1mM/1mM) respect to G1 (80,3±3,0%; $p<0,05$).

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COMUNICACIONES LIBRES II

VASCULOGENIC MIMICRY: AN ALTERNATIVE MODEL OF TUMOR IRRIGATION IN OVARIAN CANCER

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Vasculogenic mimicry (VM) occurs when cancer cells establish an alternative perfusion pathway in the absence of endothelial cells. VM strongly correlates with poor patient survival, however controversy still surrounds the existence of an *in vitro* model of VM. Despite many publications claiming to demonstrate VM *in vitro*, the majority of these studies fail to provide solid evidence of true hollow channels, raising concerns as to whether actual VM is being examined. Herein, we provide a standardized *in vitro* assay that recreates the formation of functional hollow channels using ovarian cancer spheres and primary cultures derived from ovarian cancer ascites. Fluorescence confocal microscopy, X-ray microtomography 3Dreconstruction and dye microinjection conclusively confirm the existence of functional glycoprotein-rich lined hollow structures *in vitro* and demonstrate that many of structures reported in the literature do not represent VM. This assay may aid the design and testing of future VM-blocking anti-cancer therapies.
This study was supported by FONDECYT 1160800, 11140657, 1140970, CONICYT PFB12/2007, CONICYT-FONDAP 15130011, IMII P09/016-F, and the PUC Proyecto interdisciplina II15058.

INDUCTION OF BOVINE BONE MARROW MESENCHYMAL STEM CELLS INTO MALE GERM CELLS USING TRANSFORMING GROWTH FACTOR-B SUPERFAMILY GROWTH FACTORS

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In vitro gamete derivation technology has potential applications as an alternative method for dissemination of elite animal genetics, production of transgenic animals, and conservation of endangered species. Mesenchymal stem cells (MSC) may be suitable candidates for *in vitro* gamete derivation considering their wide differentiation potential and the abundant tissues sources for isolation. The present study aimed to induce bovine fetal MSC (bfMSC) *in vitro* differentiation into the germ cell lineage using transforming growth factor-β1 (TGFβ) and bone morphogenetic protein 4 (BMP4). Differentiation media consisted in control media (DMEM with high glucose plus 10% FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B) supplemented with TGFβ1 (1, 10 or 100 ng/mL) or BMP4 (10, 50 or 100 ng/mL). bfMSC and testis samples (positive controls) were analyzed for expression of housekeeping genes β-ACTIN and GAPDH, pluripotent genes OCT4 and NANOG, germ cell genes FRAGILLIS, STELLA, and VASA, male germ cell genes DAZL, PIWIl2 and STRA8, and meiotic biomarker SCP3 by quantitative-PCR (Q-PCR). Transcripts of all genes were detected in testis with highest ($P<0.05$) levels of VASA, DAZL and PIWIL2. Supplementation of BMP4 to bfMSC induced a dose-response effect on NANOG mRNA levels. TGFβ1 and BMP4 induced up-regulation ($P<0.05$) of DAZL mRNA levels at days 7 and 14 of differentiation, respectively. OCT4 and SCP3 mRNA levels were not affected by RA or BMP4 treatments. Thus, exposure of bfMSC to transforming growth factor-β superfamily growth factors under *in vitro* conditions might induce an early stage of premeiotic germinal differentiation. Supported by Fondecyt grant 1161251, Government of Chile.

THE SYNTHETIC METALLOPORPHYRIN MnTBAP PROTECTS EQUINE SPERM CELL AFTER CRYOPRESERVATION

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During cryopreservation procedures, the spermatozoa are exposed to physical andchemical stressors that generate an increase in the level of intracellular ROS. Overproduction of ROS can lead to a state of oxidative stress that undermine sperm quality. The aim of this study was to evaluate the protective role of MnTBAP, scavenger of anion superoxide (O2-), on quality variables of equine cryopreserved spermatozoa. Frozen/thawed equine spermatozoa (2 x 106 mL-1) were incubated with 50 µM, 100 µM and 150 µM MnTBAP during 4 hours at 38°C. An untreated sperm suspension and a fresh sample were included as controls. After treatments, the sperm suspensions were washed by centrifugation and resuspended in DPBS. We evaluated viability (SYBR-14/PI), intracellular ROS level (DHE y ROS-IDä□ total ROS/Superoxide Detection Kit), lipid peroxidation (BODIPY), DNA damage (TUNEL) and mitochondrial membrane potential dissipation (D□Y□m; TMRE/SYTOX), all of them by flow cytometry. In addition, sperm motility was evaluated using the ISAS system. Evaluations were performed at 0 and 4 hours of incubation. The results showed that O2- is the main ROS produced in frozen/thawed equine spermatozoa and that MnTBAP improved significantly the sperm motility and viability, decreased the lipid peroxidation and DNA damage. Future research is needed to evaluate the effects of MnTBAP on the functionality of frozen/thawed equine spermatozoa, specifically sperm capacitation, acrosome reaction, hyperactivation and spermatozoa-oocyte fusion. This work was supported by a Postdoctoral fellowship FONDECYT (grant number 3160214) CONICYT-Chile, and FONDECYT (grant number 1160467), CONICYT, Chile.

OVARIAN REVERSE CHOLESTEROL TRANSPORT: CONTRIBUTION OF FOLLICULAR FLUID HDL AND ABCA1 TRANSPORTER TO MOUSE OOCYTE CHOLESTEROL HOMEOSTASIS

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Cholesterol efflux from cells to high density lipoproteins (HDL) is mediated by membrane transporters such as ATP-Binding Cassette transporter A1 (ABCA1) and ABCG1. HDL cholesterol is taken up by liver Scavenger receptor class B type I (SR-BI) and excreted in bile. Altogether, this process is known as reverse cholesterol transport (RCT). SR-BI deficient (KO) mice have large, cholesterol-rich HDL. Females are infertile due to high spontaneous activation and reduced viability of eggs, associated to an abnormal cholesterol content. Cholesterol accumulation in SR-BI oocytes starts in antral follicles and treating SR-BI KO mice with Probucol, a drug reducing blood cholesterol levels, prevents cholesterol accumulation in eggs. Here, we hypothesized that follicular fluid HDL permeating from plasma regulates the egg´s cholesterol content. Our aims were to analyze: 1) if plasma HDL permeate into the follicle, 2) if cholesterol transporters are expressed in eggs and 3) whether cholesterol excess in SR-BI KO oocytes is due to excessive influx or defective efflux from/to HDL. Ovaries from apoA-I (HDL marker) KO mice were transplanted into WT mice. Two weeks later, indirect immunofluorescent using anti-apoA-I showed positive staining in the antrum of apoA-I KO ovaries, suggesting that plasma HDL cross the follicular basal membrane. Expression of ABCA1 and ABCG1 (mRNA and protein) was detected in eggs by RT-PCR and indirect immunofluorescence. Finally, incubation of SR-BI KO oocytes with WT HDL normalized their cholesterol content, evaluated by filipin (fluorescent marker for cholesterol). These results support the existence of a local RCT ovarian system involving ABCA1-mediated cholesterol efflux from eggs to antral HDL.
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UNDERSTANDING CHOLESTEROL METABOLISM DURING EARLY PREGNANCY

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Cholesterol is transported in the bloodstream by lipoproteins. To regulate circulating cholesterol levels, HDL-cholesterol is taken up by liver scavenger receptor class B type I (SR-BI) and excreted into bile through specific transporters. We recently found that mouse females exhibit a strong reduction in plasma cholesterol during early pregnancy. Despite cholesterol´s key role in essential processes of embryo development, i.e. steroid hormone synthesis and membrane formation, cholesterol metabolism during early pregnancy is poorly understood. Here, we sought to understand the mechanisms regulating the temporary cholesterol drop during early pregnancy. In pregnant mice at gestational day 8 (E8), we analyzed plasma and hepatic cholesterol levels. We also determined hepatic expression of SR-BI (by western blot) and of genes involved in cholesterol synthesis, lipoprotein production, and biliary excretion (by qPCR). Compared to non-pregnant females, pregnant dams showed reduced plasma HDL cholesterol (-40%; p=0.0082), but similar hepatic cholesterol concentrations and SR-BI protein levels. Expression of genes involved in HDL synthesis (Apoa1 and Apoe) was significantly reduced in livers from pregnant vs. non-pregnant females, suggesting lower HDL formation. By contrast, expression of genes related to cholesterol synthesis (Squalene synthase and Dchr7) was increased whereas those related to cholesterol biliary excretion (Abcg5 and Abcg8) were down-regulated in livers from pregnant dams. In summary, reduced HDL synthesis in livers from E8 dams may contribute to the low plasma cholesterol levels. Further studies will be required to further describe and explain the physiological relevance of this temporary reduction in maternal cholesterol for early embryonic development. Funding: FONDECYT 1141236 (to D.B.), PhD fellowship CONICYT 21130444 and School of Medicine PMD-04/16 (to N.S.).

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POSTERS

EFFECT OF SYNTHETIC METALLOPORPHYRIN (MnTBAP) SUPPLEMENTATION ON EQUINE SPERM FUNCTION AND ZONE PELLUCIDA-BINDING ABILITY

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During cryopreservation procedures the spermatozoa are exposed to physical and chemical stressors that generate an increase in the level of intracellular ROS. Overproduction of ROS can lead to a state of oxidative stress, which undermine sperm functionality. The aim of this study was to evaluate the protective role of MnTBAP, scavenger of anion superoxide (O_2^-), on viability and functionality of equine cryopreserved spermatozoa. Frozen/thawed equine spermatozoa (2 x 10^6 mL-1) were incubated in a capacitating medium (CAP) supplemented with 100 µM MnTBAP (CAP+MnTBAP) during 4 hours at 38°C. A non-supplemented group (CAP) and non-capacitating conditions (NCC) were included as controls. After treatments, we evaluated viability and plasma membrane fluidity (Merocianine-540/Sytox green) by flow cytometry. Sperm motility was assessed using ISAS System. In addition, we performed zone pellucida-binding ability assay using bovine MII oocytes. The results showed that MnTBAP compared to CAP and NCC groups improved significantly the sperm motility (21, 16 and 9 µm/s, respectively), viability (35, 12 and 20%, respectively), and plasma membrane fluidity (15, 11 and 5%, respectively). Although zone pellucida-binding ability was similar (P>0.05) among all conditions (CAP+MnTBAP, CAP and NCC), the supplemented group showed the higher amount of oocyte-bound sperm (44, 33, 29 sperm/oocyte, respectively). Future research will be focused on fertilization ability of frozen/thawed capacitated equine spermatozoa supplemented with MnTBAP.
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RECOVERY OF THE SYMPATHETIC INNERVATION IN RAT OVARY IN PERIOD OF FERTILITY AND SUBFERTILITY

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In mammals, the ovary function is regulated concomitantly by two different mechanisms: one exerted by gonadotropins and the other by the sympathetic innervation. In the rat the superior ovarian nerve (SON) is the main ovarian sympathetic network being noradrenaline (NA) its main neurotransmitter. NA participates actively in the synthesis of androstenedione and progesterone independently of the gonadotropins. It is known in young rats that when SON is surgically sectioned, the ovary recovers the innervation after about 28 days. The aim of this work was to determine if the plasticity of these nerve fibers is maintained during the subfertile period. 5 months old (fertile period) and 9 months old (subfertile period) Sprague-Dawley rats were submitted to bilateral section of the SON (SONX) and other similar group was shame operated as control (SHAM). The rats were euthanized 28 days after surgery. To assess the recovery of the sympathetic innervation, ovarian NA and Nerve Growth Factor (NGF) and serum Estradiol (E_2) were determined. NA concentration after 28 days of denervation, is diminished (264.42 ± 81.9 in *sham* rats *versus* 136.648 ± 72.05 pg/mg ovary in *SONX rats* at 05 months of age, $p<0.05$); 228.627 ± 45.86 in *sham* rats *versus* 27.81 ± 10.64 pg/mg ovary in *SONX28* rats at 9 months of age, $p<0.05$. While the levels of NFG (21.92± 4.86 ng/mg ovary in rats 5 months of age and 28 ± 6.56 ng/mg ovary in rats 9 months of age) and serum E_2 (182.06 ±14.2 pg/ml in rats of 5 months of age and 211.03 ± 16.39 pg/mL in rats of 9 months of age) do not shown variations. These results suggest that with the increase of the reproductive age the capacity of reinnervation of the ovary is disminished. FONDECYT 1120147

EVALUATION OF HTF MEDIUM AS HANDLING AND CAPACITATION MEDIUM FOR EQUINE SPERMATOZOA: PRELIMINARY RESULTS

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One of the most widely used culture media for handling, incubation and capacitation of equine spermatozoa has been the Whitten's medium, however, its success on *in vitro* capacitation of spermatozoa is still low. The objective of the present study was to evaluate the performance of the HTF medium, mainly used in human and mice, as handling, and incubation medium of equine spermatozoa. For this, fresh semen from 4 Chilote breed stallions was used and diluted to $10x10^6$ sperm/mL in no capacitating (NC) and capacitating (C) conditions and incubated for 30 and 120 minutes at 38°C in air atmosphere, using the Whitten's medium as control. Plasma membrane integrity (SYBR/PI), mitochondrial membrane potential (TMRM/Sytox), membrane phospholipids disorder (MC540/Sytox) and acrosomal membrane integrity (PNA-FITC/PI), were evaluated trough flow cytometer. Preliminary results show no significant differences in quality and sperm functionality parameters evaluated between both media at 30 and 120 minutes of incubation. Additionally, it was confirmed that HTF medium, as the Whitthen's medium, maintained a slight increase of the pH during the time, which has been recently observed that it is beneficial for the sperm capacitation in equine. These results indicate that the HTF medium can be used for the handling and incubation of equine spermatozoa, since it does not affect the functionality and sperm quality and maintains the desired pH during the sperm incubation. Further experiments are currently underway to evaluate the effect of this medium on the sperm motility and hyperactivation.
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EVALUATION OF THE EFFECT OF CHRONIC TESTOSTERONE ADMINISTRATION ON INSULIN SENSITIVITY (IS) IN ADULT OOFERECTOMIZED SHEEP EXPOSED PRENATALLY TO AN EXCESS OF TESTOSTERONE (EPT)

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EPT causes a decrease in IS in ewes from 5 weeks of age, which is later accentuated during adult life. Previous studies in EPT ovine females, treated chronically with testosterone (T) to determine if T acting as a potentiator of the T reprogramming effect, showed a decrease in IS. However, to assess the role of T as a trigger for insulin resistance, independent of fetal reprogramming, IS was assessed by the intravenous glucose tolerance test (IVGTT) in ooferectomized EPT sheep after chronic administration of T. The EPT sheep model consisted of the administration of T, from day 30 to 120 of gestation, to pregnant sheep. The offspring (C-females and T-females, n = 6 in each group) were ooferectomized at 26 weeks of age. At 30 weeks, they underwent a T-protocol for 8 weeks and the IVGTT was performed. Body weight, plasma concentration of testosterone, estradiol and progesterone were similar between groups. The dynamics of the plasma concentration of insulin and glucose during TTGEV showed no differences. The IS indexes, estimated from the insulin and glucose concentration during IVGTT, were similar in both groups. However, the glucose disappearance rate was higher in the T-females ($p<0.05$). Therefore, chronic administration of testosterone in ooferectomized EPT females, independent of steroids produced and secreted by ovaries, does not modify the sensitivity of peripheral tissues to insulin.
Project FONDECYT#1140433 and Laboratory of Animal Physiology and Endocrinology (FISENLAB), Department of Animal Science, Faculty of Veterinary Sciences, Universidad de Concepción, Campus Chillán.

EVALUATION OF THE EFFECT OF PRENATAL EXPOSURE TO TESTOSTERONE (EPT) ON MORPHOMETRIC PARAMETERS AND PLASMA CONCENTRATIONS OF HORMONES AND GLUCOSE IN FEMALE OVINE FETUS AT 120 DAYS OF GESTATION

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Polycystic Ovarian Syndrome (PCOS) is an endocrine-metabolic pathology present in approximately 10% of women of reproductive age, with a strong epigenetic and probably transgenerational component. The hyperandrogenic uterine environment, in which the fetus develops, would be involved in the susceptibility of daughters to PCOS, along with other cardiovascular, reproductive, endocrine and metabolic disorders. The sheep model of PCOS has provided a sustenance to this hypothesis. In our model we evaluated the anatomical morphometry and endocrine alterations induced by EPT in female fetuses at 120 days of gestation (dg). For this, testosterone was administered in pregnant females between 30-120 dg. T-fetuses and C-fetuses (n=17 and n=19, respectively) were collected, euthanized, morphological parameters were recorded and blood samples were obtained from the jugular vein for the determination of hormones and glucose*. T-fetuses had a shorter total length than C-fetuses (P=0.04). In the T-fetuses an urethral elongation (635%) and generation of a pseudoescrotus were observed. The

weight of the liver, pituitary, epiphysis, ovaries and left adrenal gland were similar between the experimental groups, but the weight of the right adrenal gland was lower and the weight of the pancreas tended to be lower in the treated group. Plasma concentrations of IGF-1 were lower in T-fetuses; Testosterone and estradiol were lower in C-females ($P<0.05$). Plasma glucose concentration was similar between both groups. These data suggest that EPT would induce some anatomical and endocrine alterations, in the T-fetuses, that would increase their risk of certain pathologies during adult life.
* This protocol was revised and approved by the Bioethics Committee of the Faculty of Veterinary Sciences of the Universidad de Concepción.
Project FONDECYT#1140433 and Laboratory of Animal Physiology and Endocrinology (FISENLAB), Department of Animal Science, Faculty of Veterinary Sciences, Universidad de Concepción, Campus Chillán.

TESTOSTERONE ADMINISTRATION DECREASES INSULIN SENSITIVITY (IS) IN ADULT FEMALE SHEEP BORN TO PREGNANT TESTOSTERONE TREATED MOTHERS

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Different animal models have been used to study the postnatal effect of a prenatal exposure to an androgen excess on the female offspring. Previous results from our laboratory have demonstrated that female sheep born to mothers receiving testosterone (T) during part of their pregnancy exhibit features from early postnatal life until adulthood resembling those of PCOS women. In the present work the programming effect of prenatal T on the IS was explored in adult females born to T treated mothers (T-females) which were injected chronically with testosterone. Our aim was to establish if exogenous T may exacerbate the insulin resistance due to programming effect of prenatal exposure to T through intravenous glucose tolerance test (IVGTT). Both groups were injected with T, twice weekly (40 mg per dose), for 8 weeks, beginning at 30 weeks of age. On the day of the IVGTT, there was no difference in plasma concentration of estradiol, progesterone and T between groups. IS indexes were calculated with the plasma insulin and glucose concentrations during the IVGTT. Plasma levels of glucose were not different during the IVGTT but T-females secreted more insulin ($P<0.05$) than C-females. The ratio insulin/glucose before the IVGTT tended to be higher in T-females ($P=0.054$) and the IS Index-C tended to be lower in T-females compared to C-females ($P=0.078$). Results show that T administration to T-females amplifies the effect of a glucose challenge on the insulin secretion compared to C-females, suggesting an exacerbation of the insulin resistance induced by fetal programming.

Project FONDECYT#1140433 and Laboratory of Animal Physiology and Endocrinology (FISENLAB), Department of Animal Science, Faculty of Veterinary Sciences, Universidad de Concepción, Campus Chillán.

PRENATAL METFORMIN TREATMENT PREVENTS ESTRADIOL INCREASE AND PARTIALLY IMPROVES OVARIAN FUNCTION IN THE OFFSPRING OF OBESE RATS

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Maternal obesity is related to metabolic and reproductive dysfunctions in the offspring. We previously demonstrated that female offspring of obese rats have increased estradiol levels during early postnatal life, which probably induces an early onset of puberty and a polycystic ovary condition during adulthood. The obesogenic microenvironment during gestation leads to early onset hepatic dysfunction associated to a decrease in estradiol metabolism through the enzyme CYP3A2. In the present work, we improved the metabolic status of the mother by using the drug metformin during gestation and nursing with the hypothesis that this treatment prevents the sequence of events conducing to the increase in postnatal estradiol levels in offspring, thus preventing the development of reproductive impairments. Our results show that metformin administration to the mothers prevented the increase in estradiol levels at postnatal day (PND) 14 and PND60 in offspring of obese mothers, events associated with a restoration of hepatic CYP3A2 to control levels. The advanced puberty was not prevented by the treatment with metformin, but we observed that the number of antral follicles, follicular cysts and multioocyte follicles returned to control levels in the offspring of rats treated with metformin. Additionally, we observed an increase in norepinephrine (NE) content and in the NE metabolite 3-Methoxy-4-hydroxyphenylglycol (MHPG) in the ovary, indicating an increased sympathetic activity induced by the obesogenic uterine environment, effect that was prevented by gestational metformin administration. Altogether, we conclude that metformin treatment during gestation partially prevents the ovarian impairments in the offspring of obese mother during adulthood. FONDECYT 11130707 to G.C.

SPERM OF PINK CUSK-EEL (GENYPTERUS BLACODES, SCHNEIDER 1801: EFFECT OF PH, OSMOLALITY AND TEMPERATURE ON SPERM MOTILITY

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Pink cusk-eel (Genypterus blacodes) is a native Chilean species. However, few studies have addressed the effects of osmolality, temperature and pH on sperm motility of this species. In this work, spermatozoa were diluted in StorFish® medium and motility was assessed subjectively at different pH values, temperature, proportions of sea water and distilled water. Sperm motility was measured as the percentage of motile cells and velocity according to the 1 to 5 scale: 1 = 0-5%; 2 = 5-25%; 3 = 25-50%; 4 = 50-75%; 5 = 75-100%. Our results show that intratesticular spermatozoa of Genypterus blacodes have sperm density of 5.35 ± 0.16 x 109 spermatozoa/mL. Sperm motility is initiated in contact with a hyperosmotic swimming medium, and under normal

conditions (1010 mOsm/kg, pH = 8 and 8°C). However, a pH higher or lower than 8.0 has a negative effect on both percentage and duration of motility. The longest motility duration (432.48 ± 8.89 s) was recorded at 4°C. However, regardless of assessed temperatures, a gradual decrease in motility was observed at osmolalities of 772 mOsm/kg and 448 mOsm/ kg, whereas immobility of spermatozoa was observed at 0 mOsm/kg. The maximum percentage of motile cells was registered at 8°C (65.66 ± 4.95) with normal osmolality (Control: 1010 mOsm/kg), whereas an optimum was observed at pH 8.0. Our results shown that the flagellar activity is initiated by hyperosmotic medium and 4ºC is a optimal temperature to induced an increase on sperm motility in Pink cusk-eel (Genypterus blacodes). Acknowledgments: This work was financially supported by FONDECYT Project No. 1151315 (JF) and Universidad de la Frontera Doctorate Scholarship (KD).

GLOBAL DNA METHYLATION SINCE EARLY INFANCY TO ADULTHOOD IN GIRLS AND BOYS BORN TO WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

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DNA methylation is an epigenetic mechanism of gene regulation that can be modified during intrauterine and postnatal life. Pregnant women with polycystic ovary syndrome (PCOS) present elevated androgen and insulin levels, which can affect the DNA methylation pattern of their offspring. Then, we studied the global DNA methylation pattern (GDM) in daughters and sons born to PCOS women compared with those born to non-PCOS women. Daughters (99 born to PCOS and 87 born to control women) and sons (74 born to PCOS and 93 born to control women) at early infancy (2-3 months), puberty (7-17 years) and adulthood (18-35 years) were included. In all of them, a clinical-anthropometric examination was performed and a blood sample was obtained for DNA isolation from peripheral leukocytes. The absolute percentage (%) of GDM was quantified using a colorimetric kit (Epigentek). In sons, PCOS and control showed changes in GDM from early infancy to adulthood ($p<0.001$ PCOS and $p=0.045$ control, ANOVA test). Moreover, sons born to PCOS presented lower GDM compared to sons born to controls in early infancy (3,0% vs 7,4%, $p=0,043$) and at the beginning of sexual maturation (2,9% vs 7,1%, $p=0,010$). In daughters, no differences in GDM pattern from early infancy to adulthood or between PCOS and control groups were observed. Our data indicate that males seems to be more susceptible than females to changes the GDM, mainly in periods of activation of gonadal axis, as early infancy and beginning of puberty.
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EFFECT OF THE ADDITION OF DIBUTYRYL-AMPC (DBAMPC), 3-ISOBUTIL-1-METHYLXANTHINE (IBMX) AND METHYL-B-CYCLODEXTRIN (MβCD) TO THE WHITTEN´S MEDIUM ON THE QUALITY OF EQUINE SPERM

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The events involved in capacitation lead the sperm to changes in the pattern of motility and acrosomal exocytosis that allow the penetration of the oocyte´s pellucid zone and finally the fertilization. While under appropriate laboratory conditions spermatozoa of different mammalian species have been capacitated *in vitro*, optimal conditions for sperm capacitation in the equine species that allow high protein tyrosine phosphorylation and acrosomal exocytosis have not been

found. In the present study, we assessed the effect of the addition to the Whittens medium of different sperm capacitation inducers (dbAMPc, IBMX y MβCD). For this, equine spermatozoa at a concentration of 10x106 sperm/mL were incubated in this medium containing different concentration of the capacitation inducers for 4 h at 38 °C in an air atmosphere. Sperm viability (PI), mitochondrial membrane potential (TMRM/SYTOX Green), acrosomal membrane integrity (PNA-FITC/PI) and the degree of membrane fluidity (MC540/SYTOX Green) were analyzed by flow cytometry. Preliminary results confirm that these treatments do not affect the sperm viability nor the mitochondrial membrane potential with the exception of the highest concentrations of IBMX and MβCD evaluated. Additionally, the treatments evaluated did not affect the acrosomal membrane integrity while only the treatments with MβCD showed a greater plasma membrane fluidity, attributed to its effect as cholesterol acceptor. Future experiments currently are underway to confirm the effect of these inducers in other sperm capacitation markers and motility parameters.
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CHRONIC EXPOSURE TO A MIXTURE OF PHTALATES AND ALKYLPHENOLS MODIFIES AROMATASE (*Cyp 19a1*), ESTROGEN RECEPTOR *Erβ* and Pre-MicroRNAs MODULATING LEVELS IN SPERMATOZOA, AFFECTING ACROSOME REACTION (AR) AND FERTILIZATION IN MOUSE

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Phthalates and alkylphenols are environmental pollutants that modulate spermatogenesis events, altering the action of estrogen and microRNAs. Therefore, our goal was to investigate whether the chronic administration of a mixture of these compounds modifies the expression levels of the aromatase enzyme (*Cyp19a1*) and the *Erβ* receptor, via the deregulation of microRNAs that modulate them, in mouse spermatozoa. For this, male mice were treated with a mixture of these compounds from gestation until two months of life. These mice had a 40% reduction in birth weight and the sperm concentration and the testosterone / estradiol ratio were reduced by 50% compared to mice treated with vehicle. The total sperm [RNA] ng/106 of the treated mice was increased 2.5 fold and the levels of aromatase enzyme mRNA and the Erβ receptor mRNA were increased 2.6 and 32 fold to the control, respectively. Concomitantly, the levels of both proteins increased 7.7 and 32.6 times. On the other hand, using cell lines derived from male germ cells, we found a decrease in 1.3; 1.7; 6 and 3 times the levels of the premicroRNAs 200b, 7b and 7g, which target the aromatase mRNA and and *Erβ,* which could explains the increase observed in these messengers. Moreover, exposure to this mixture has important biological effects since the spermatozoa of exposed mice experience 20% more progesterone-induced RA and 22% less in the rate of *in vitro* fertilization. In conclusion, this mixture of phthalates and alkylphenols affects the mRNA levels in the spermatozoon, fertilization and could affect the embryo.
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EFFECTS OF PRENATAL EXPOSURE TO AN EXCESS OF TESTOSTERONE (EPT) ON STRUCTURAL DEVELOPMENT AND MATURATION OF PULMONAR TISSUE IN FEMALE SHEEP FETUS AT 120 DAYS OF GESTATION

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During fetal life the correct development of respiratory structures, together with the adequate production of pulmonary surfactant (SP), allow the lung to reach the maturity for life after birth. However, the lung can be diverted from its normal developmental pathway. The objective of the study was to evaluate the effect of EPT on the structural development of the lung and the expression of the mRNA of the SP proteins. The study was performed on an EPT sheep model, which consists of the administration of testosterone in pregnant ewes from the day of gestation 30 to 120. Once the protocol was completed, the fetuses were removed, the lungs were weighed and a sample from the left caudal lobe was collected (T-fetuses and C-fetuses, n=5 per group) to evaluate histological structure by morphological analysis and RNAm expression of SP proteins by qPCR. There were no differences in body weight and lung weight. Likewise, the morphological parameters were similar in both experimental groups ($p<0.05$). However, there was a decrease in the expression of SP-A and SP-B in the T-fetal group when compared to the C-fetus (p0.05). Therefore, EPT would not modify the lung morphology, but would negatively affect the expression of proteins that are part of the SP causing functional and immunological alterations that would increase the predisposition to respiratory pathologies after birth.
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ESTROGEN METABOLITES IN THE REGULATION OF ANGIOGENESIS IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME

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Angiogenesis is an important factor of development, selection and subsequent follicular rupture. At this stage the estrogens biosynthesis and their metabolites exert a modulating effect on angiogenesis. It is plausible that Follicular arrest of Polycystic Ovary Syndrome (PCOS) may be due to failure in the process of ovarian angiogenesis. This investigation determines the levels of pro-angiogenic estrogen metabolites (EM), 16-ketoestradiol, 2-hydroxyestradiol and 4-hydroxyestrone in normal and PCOS women. In addition we study the effect on the angiogenic potential of human granulosa cell culture (GC). EM levels were determined in follicular fluid (FF) of normal women requesting tubal sterilization and in PCOS women that need ovarian drilling, by HPLC coupled to mass spectrometry. The angiogenic potential (AP) was evaluated by in vitro angiogenesis assays of conditioned media of luteinized CG culture from normal patients and PCOS women participating in the IVF program. The levels of pro-angiogenic EM were lower in FF of PCOS patients compared to fertile women ($P <0.05$). The angiogenic potential of GC culture under basal conditions and hCG stimulated, were lower in PCOS patients compared to normal women ($P <0.05$). The finding of reduction in pro-angiogenic EM levels in FF of PCOS patients would partially explain the reduction in the angiogenic potential of GCs of PCOS patients. These data suggest a deregulation of angiogenesis in altered follicular development of PCOS.
FONDECYT REGULAR 1140693

LEVELS OF MICRORNA 23B IN OVARIAN TISSUES AND THE EFFECT OF NGF IN THE LEVELS OF THIS MICRORNA IN EPITHELIAL OVARIAN CELL LINES

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Epithelial ovarian cancer (EOC) is characterized by being diagnosed in advanced stages and a poor response to therapy. The levels of Nerve Growth Factor (NGF) and its receptor TRKA are both elevated in EOC, being important in its progression. Currently, microRNAs are involved in different pathologies including cancer; among them microRNA-23b is diminished in a large number of tumors and in EOC the low levels are associated with more aggressive tumors and a low survival. The aim of this study was to determine the levels of miR-23b in normal ovary, ovarian tumors and EOC and to evaluate whether NGF regulates the levels of this miR in ovarian cell lines. The patients underwent hysterectomy with oophorectomy at Clinical Hospital, University of Chile, previously a signed informed consent, approved by the Institutional Ethics Committee. The samples were classified into inactive ovary (IOV, N=6), ovarian tumors (OT, N=7) and EOC (N=10). Furthermore, human normal ovarian epithelial (HOSE) and epithelial ovarian cancer (A2780) cell lines were used. The levels of miR-23b were evaluated by Real Time PCR. The results showed that microRNA-23b decreased 51% in the ovarian tumor and 50% in COE when compared to OVI ($p < 0.01$). The levels of miR-23b in A2780 cells decreased 99% compared to HOSE cells ($p < 0.05$). MicroRNA-23b levels decreased in 50% and 59% in HOSE and A2780 cells, respectively when cells were treated with NGF ($p < 0.05$); this effect was reverted when antibody against NGF and TRKA inhibitor were used. These results suggest that NGF could play an important role in decreasing miR-23b levels during EOC progression.
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ADDITION OF THE ANTIOXIDANT BUTYLATED HYDROXYTOLUENE IN THE FREEZING MEDIUM OF CAT SPERMATOZOA: EVALUATION OF EFFECT ON SPERM MOTILITY AND VIABILITY

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Butylated hydroxytoluene (BHT) is a most commonly used antioxidant as additive in industrial applications. Similarly, it has been used in the freezing media in semen of several species of mammalian (turkey tom, stallion, buffalo, boar, goat and bull) and fish, especially to prevent the alterations in plasma membrane during the freezing due to its great antioxidant capacity. However, to our knowledge, there are no reports about of its use in the freezing medium in cat semen and their effects on sperm quality. The aim of this study was to determine the effect of BHT added in different concentrations to the extenders of freezing on semen parameters of motility, viability and membrane integrity in the cat spermatozoa. A total of 7 ejaculates from four cat were collected by electroejaculation. Eight replicates of the ejaculates were frozen in a egg yolk Tris-fructose-citric acid (EYT-FC) +7% glicerol+1% Equex STM Paste based extender, supplemented with different concentrations (0,0 mM; 0,5 mM; 1.0 mM and 2.0 mM) of BHT. Post thawing semen, sperm membrane integrity using flow cytometry and motility using a computerized system were evaluated. Addition of BHT (0,5 mM and 1.0 mM) tended to increase sperm total motility, viability and membrane integrity post-thaw compared to the control, however, observed differences were not statistically significant ($p>0,05$). In conclusion, BHT can be used in cryopreservation of cat semen in order to reduce damage to the plasma membrane on spermatozoa. However, it is important to continue the research to delimit the adequate concentration of BHT that protects sperm quality during freezing.
DIUFRO N° DI17-0092**;** FONDECYT POST-DOCTORADO N°3150320.

AMP-ACTIVATED PROTEIN KINASE (AMPK) ACTIVITY IS REDUCED IN SPERMATOZOA OF *SALMO SALAR* AFTER MOTILITY ACTIVATION AND UNDER CRYOPRESERVATION CONDITIONS

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Sperm motility lasts for short time in freshwater fish species and is strongly affected by cryopreservation, with direct impact in fertilizing capacity. Cryodamage has been studied focusing on mitochondrial function, oxidative stress and membranes lipid composition. However, few studies have addressed this issue from energetic and signalling points of view and several aspects of molecular mechanisms of motility activation in fish spermatozoa remain unclear. Current research are focused on membrane signal transduction, molecular mechanics of flagella, and energy regulation. In this work, content and activity of AMPactivated protein kinase (AMPK) were measured in fresh non-activated (control), activated, and cryopreserved spermatozoa of *Salmo salar*. High-quality samples were used, exhibiting high percentages of cells with intact plasma membrane, high mitochondrial membrane potential (> 95%) and motility parameters (>90 % of motile cells). Cryopreserved samples displayed significantly lower values and motility was completely suppressed. Nevertheless, a non-negligible amount of cells (25% approximately) remained viable, but incapable to become motile. Total AMPK levels showed no significant difference among samples, while phospho-AMPKThr172 (active form) significantly decreased after activation and cryopreservation compared to control. In conclusion AMPK is expressed in spermatozoa of *S. salar* and its active form significantly decreases 15 seconds after motility activation; cryopreservation strongly inhibit AMPK activity along with marked reduction of motility, cell viability and mitochondrial functionality. These preliminary results suggest that AMPK is involved in motility activation processes in spermatozoa of *S. salar*, however, it role is far from being elucidated and further study is ongoing.
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PRELIMINARY ANALYSES OF CELL DEATH PATHWAYS ACTIVATED BY EXCESS CHOLESTEROL IN MOUSE OOCYTES

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High density lipoprotein (HDL) cholesterol is taken up by liver Scavenger Receptor Class B type I (SR-BI), and subsequently metabolized in bile. SR-BI deficient (SR-BI KO) mice have large, cholesterol-rich HDL. Females are infertile due to cholesterol excess and reduced viability of eggs. We previously failed to detect markers of apoptotic damage in SR-BI KO eggs, suggesting that lability is due to activation of other death pathways. Excess lipids in oocytes from obese mice has been shown to induce damage in mitochondria and endoplasmic reticulum (ER), leading to autophagy whereas in some cells, cholesterol excess associates to high reactive oxygen species (ROS) and mitochondrial dysfunction, associated to lower viability. Here, we evaluated ROS levels, mitochondrial function and markers of autophagy in eggs with excess cholesterol. We used SR-BI KO eggs and WT eggs loaded with cholesterol by incubation with MβCD-cholesterol, as we demonstrated that these cholesterol-enriched eggs also exhibit a compromised viability. Both cholesterol-loaded eggs and SR-BI KO eggs showed similar ROS levels than WT eggs, evaluated by diclofluorescein diacetate fluorescence. Cholesterol-loaded oocytes incubated with JC-1 fluorescence probe, a marker of the mitochondrial membrane potential, showed increased red/green fluorescence ratio, suggesting mitochondrial hyperpolarization. Compared to WT eggs, SR-BI KO eggs exhibited higher fluorescence for LC-3, a protein involved in the formation of autophagosomes, by indirect immunofluorescence, and larger autophagosomes, detected by transmission electronic microscopy. Future studies will be designed to analyze if mitochondrial dysfunction and autophagy contribute to the low viability observed in SR-BI KO eggs.
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ACTIVATION OF INTERLEUKIN-6 (IL-6) PATHWAY IN ENDOMETRIA OF WOMEN HAVING POLYCYSTIC OVARIAN SYNDROME (PCOS)

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PCOS is an endocrine and metabolic disorder highly prevalent in women in reproductive age, characterized by hyperandrogenism and ovarian dysfunction. Approximately, 80% of PCOS-women are obese, indicating the presence of a pro-inflammatory microenvironment. Reports have shown an increase in IL-6 blood levels of obese PCOS-women. One of the signaling molecules of IL-6 action is STAT3. Therefore, the aim of this study was to evaluate the activation of STAT3 in endometria obtained from PCOS-women. For this, endometria from 4-groups of PCOS-patients were studied (n=12): Normal-weight, Lean-PCOS, Obese, Obese-PCOS. Levels of IL-6, IL-6-Receptor, STAT and p-STAT3-Tyr705 were determined by immunohistochemistry (software Image ProPlus 6.2) and IOD (integrated optical density) was obtained and analyzed by Kruskall-Wallis/Dunn-test. The results show that the immunostaining of all proteins assessed was homogenously distributed in the cytoplasm. Endometrial levels of IL-6 were lower in Normal-weight endometria compared to other groups ($p<0.05$), whereas, IL-6-Receptor was similar in all groups. Besides, the phosphorylated fraction of STAT3 (p-STAT3/STAT3) was higher in endometria of Lean-PCOS vs Normal-weight ($p<0.01$) and in Obese-PCOS compared to Obese ($p<0.05$). Moreover, activation of STAT3 was higher in Lean-PCOS compared to Obese-PCOS ($p<0.01$). These data indicate that the signaling pathway of IL-6 is active in human endometrial cells, suggesting a pro-inflammatory environment in these cells, being higher in both PCOS-groups. This is in agreement with the fact that STAT3 exerts an activation of androgen receptors. Even more, the finding that the activation of STAT3 is higher in Lean-PCOS than in Obese-PCOS probably reflects the negative action of insulin in IL-6 action, as reported in hepatic cells.
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ANTIMiRs AGAINST Mir-7013-5P, Mir-7116-5P AND Mir-6373 generate DIFFERENTIAL EFFECTS ON THE TRANSLATION OF THE REPORTER GENE EGFP FUSED TO *Rnf19A* OR *Rnf19A-TPPG3* 3'UTRs IN SOMATIC VERSUS GERM CELL LINES

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MicroRNAs (miRNAs) are small regulatory RNAs that function in a net of interactions with target mRNAs controlling post-transcriptionally their decay and translation. MiRNA's prevalence and activity are adjusted in turn by numerous regulatory mechanisms. Many reports show that miRNAs are themselves targeted by RNA species that regulate them. Several classes of noncoding RNAs, such as pseudogene transcripts, have been identified. These species carry miRNA binding sites becoming miRNA "sponges" that competitively sequester miRNAs from their natural targets. In a previous work, we demonstrated that fusing EGFP coding sequence to either *Rnf19a* gene 3' UTR or the one from its pseudogene, *Rnf19aTPPg3* 3' UTR generates a decreased EGFP fluorescence when transferred into cells. To prove that this decrement is generated by miRNA binding, we co-transfected antimiRs against 3 selected miRNAs together with the previously used constructs. The antimiRs were designed against miR-7013-5p; miR-7116-5p and miR-6373 which were predicted to be common regulators of *Rnf19a* gene and the three of its pseudogenes. These three miRNAs have also been reported as to be expressed in testicular tissue.
When somatic NIH3T3 cells were co-transfected with any of the antimiRs and both constructs previously mentioned, we show a recovery of EGFP expression to control levels. However, when the same experiment was performed on germ cell derived GC-2 cell line, we show a decreased in EGFP fluorescence intensity. These unexpected results obtained in germ cells may highlight a different regulatory network in which both *Rnf19a* gene and its pseudogene 3 are involved in these cells.

DISTRIBUTION AND EXPRESSION OF ANDROGEN RECEPTOR AND 5 ALPHA REDUCTASE II ENZYME IN HUMAN EPIDIDYMIS

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Androgens are hormones required for the development of the male reproductive system and secondary sexual characteristics. The epididymal microenvironment plays a fundamental role in the acquisition of the fertile capacity of spermatozoa. The functional importance of regionalization anatomical from the epididymis has been recognized, as the several anatomical regions have different patterns of gene andprotein expression, in agreement with the different functions relative to sperm storage and maturation. In this work, by immunohistochemistry, the expression and localization of the androgen receptor (RA) and 5 alpha reductase II enzyme were determined in histological sections from different regions of the human epididymis obtained by therapeutic orchiectomies of patients diagnosed with prostate cancer.
RA showed a differential expression in the different epididymis regions displaying a higher expression in the head and tail compared to the epididymal body. The intracellular localization of RA was mainly nuclear in all regions studied. 5 alpha reductase II enzyme presented a similar distribution and expression. RA and 5 alpha reductase II enzyme are regionalized in the human epididymis which could explain the differential functionality of this organ.
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LUTEAL STEROIDOGENESIS AND PROGESTERONE PRODUCTION IN *LAGOSTOMUS MAXIMUS*: DOES PROLACTIN PLAY A CENTRAL ROLE BY REGULATING THE EXPRESSION OF THE ENZYMES 3BETA-AND 20ALPHA-HSD?

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Prolactin (PRL) is a pleiotropic factor that modulates a myriad of processes, many of them directly affecting reproduction. In rodents, the absence of PRL causes infertility due to a failure of the corpora lutea in synthesizing and secreting the necessary amounts of progesterone (P4) to successfully sustain embryo implantation. We studied the expression of pituitary PRL, ovarian LH, P4 and PRL receptors (LHR, PR and PRLR), and of the enzymes 20α-HSD and 3ß-HSD in *Lagostomus maximus*throughout early-, mid-and term-pregnancy (EP, MP and TP), lactation (LCT) and after weaning of the litter (NP). As expected to occur in view of a forthcoming offspring, pituitary PRL increased during pregnancy and, although it decreased after parturition, mRNA levels in LCT were higher than in NP females. Luteal PRLR reached its maximum at MP and it drastically decreased at TP. LHR expression exhibited the same profile than PRLR. Maximal values of both P4 and LH were also recorded at MP. Remarkably, whereas ovarian 3ß-HSD exhibited an expression pattern similar to that of PRLR/LHR, 20α-HSD behaved in a complete opposite fashion: 20α-HSD displayed low expression at EP and MP but it reached its maximum at TP, when PRLR/LHR/3ß-HSD were minimal and coincided as well with the significant drop of circulating P4 which ultimately triggered parturition. In conclusion, both PRLR and LHR expressions would be defining the success of a gestation at term by modulating the levels of both 20α-HSD and 3ß-HSD, which ultimately determine the level of circulating P4
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IDENTIFICATION OF GENES RELEVANT FOR NEURAL TUBE CLOSURE IN SR-BI-DEFICIENT EMBRYOS USING RNA-SEQ

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Scavenger Receptor Class-B Type-1 (SR-BI) binds HDL and leads to bidirectional exchange of lipids. SR-BI is expressed exclusively in the maternal-fetal interface during early murine development. SR-BI KO embryos exhibit severe vitamin E deficiency, and around 50% show cephalic neural tube defects (NTD). To gain insights into the molecular mechanisms responsible for NTD in SR-BI KO embryos, we determined changes in transcriptomic signatures associated with this phenotype in wild-type embryos, normal KO embryos (nKO) and KO embryos with NTD (NTD) at embryonic day 9.5. The mRNA levels of expressed genes were analyzed using RNA-Seq and bioinformatic analyses. Differentially expressed genes (n=1054) were categorized using Gene Ontology terminology and validated using qPCR. Genes down-regulated in NTD embryos compared to WT and nKO were related to central nervous system development, i.e. transcription factors regulating neurogenesis. The reduced expression of neurodevelopmental genes Alx1, Alx3, Neurog2, and Pax3 in NTD SR-BI KO embryos was validated using qPCR. Up-regulated genes in NTD embryos vs. the other two groups were enriched in genes related to lipid metabolism, including protein components of HDL and other HDL receptors. Interestingly, maternal supplementation with vitamin E prevented NTD and normalized the expression of Alx1, Alx3 and Pax3. Since vitamin E is a biological antioxidant, we evaluated expression of genes regulated by oxidative stress in the transcriptomic dataset. We found differentially expressed oxidative-stress related genes in NTD embryos, related to cell cycle regulation and nutrient transport. In summary, our analysis revealed candidate genes that may explain vitamin E-deficiency associated NTD in SR-BI-deficient embryos.
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SEASONAL VARIATION IN SEMEN CHARACTERISTICS, ROS PRODUCTION AND TESTOSTERONE IN RAMS

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In this study, we analyzed the factors involved on the ram reproductive activity during breeding seasons (febrary to may) and out breeding season (august to november) in southern Chile (38º Lat. South). Six Romney Marsh rams were used in order to assess their semen production and quality, and some reproductive parameters. Live weight, scrotal circumference, body condition score, semen characteristics, such volume, sperm concentration, viability were evaluated every 15 days. Plasmatic testosterone concentration after GnRH injection was measured by radioimmunooassay monthly. Live weight and BCS was similar during the experience. Semen characteristics and quality show a clear response to the photoperiod (short days). The variables related to the quality and quantity of sperm showed significant differences over time, the highest values were obtained between the months of April and May, indicating that most seasonal reproductive capacity in this breed occurs in these months. Testosterone concentration and scrotal circumference also presented high values during these months. The maximum concentration of testosterone measured 2 hours after stimulation with GnRH, also shows higher values in the months of May and June (51 and 43 nmol / L), then these values decline at average concentrations of 14 nmol / L. Testosterone level was significantly correlated with testosterone and scrotal circumference. We concluded that Romney Marsh rams present a maximum reproductive capacity from April to June in south hemisphere (38º lat South).
Research Project: DIUFRO DI16-0065

MELATONIN DECREASES PROLIFERATION AND INVASION OF SPHERES DERIVED FROM CF41.Mg CANINE MAMMARY CARCINOMA CELLS

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Mammary cancer is a common disease affecting female dogs, and shows many similarities with breast cancer in humans. It has described the existence of a subpopulation of cancer cells with stem cell-like features (CSC), which can form spheres, resist conventional antitumor treatments explaining in part the recurrence of some cancers. We have previously described that spheres derived from CF41.Mg canine mammary carcinoma cells exhibit some stemness features. Melatonin has shown antitumor effects on cancer mammary cells; nevertheless, its effects has been poorly evaluated on CSC, especially in canine mammary cancer. Recent reports show that melatonin modulates the expression of proteins related to epithelial-mesenchymal transition in breast CSC, such as E-cadherin, vimentin and OCT-4. The aim of this study was to determine the effects of melatonin on proliferation and invasion of spheres derived from CF41.Mg cells. CF41.Mg cells were grown in DMEM high glucose supplemented with FBS and L-glutamine. CF41.Mg-spheres were cultured in ultra-low attachment plates with serum-free DMEM/F12 in presence of EGF, bFGF, insulin, B27 and heparin. Cell proliferation (MTS reduction) and invasion (transwell) assays were conducted in presence of melatonin (0.01, 0.1 or 1 mM). Melatonin induced an antiproliferative effect at 1 mM ($P<0.05$), however the effect on spheres was higher ($P<0.05$) than in parental cells. Cell invasion was inhibited in response to non-cytotoxic concentrations of melatonin ($P<0.05$) both in spheres and in parental cells. These results indicate that melatonin plays a role in the proliferative and invasive activity of CF41.Mg-CSC, representing a valuable potential agent against mammary CSC.

EFFECT OF BARLEY DEHYDRIN (P-80) ON POST-THAWING SPERM QUALITY OF ATLANTIC SALMON (Salmo salar)

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Cryopreservation is a technique in which cells remain viable over time, methodology of great importance in the aquaculture industry. But this technique has some disadvantages because induce lesions in fish sperm which in turn affect sperm quality in terms of fertilization ability, motility, DNA integrity and membrane integrity. In Chile, cryopreservation is used to preserve germ cells of high-value marine species, such as the case of salmon, because through it is able to reduce maintenance costs of breeding organisms. There are cryoprotective proteins, such as dehydrins which have positive effect on some enzymes preserving effect. This work focused on obtaining dehydrins P- 80, from barley (Hordeum vulgare) and assesses their impact as a potential sperm cryoprotectant Atlantic salmon; alternatively improve cryopreservation procedure and reduce the loss of sperm quality due to freeze-thaw process. In this study it was possible to identify the presence of the P-80 protein in the barley variety Scarlet cold acclimatised. The results of this study show that treatment with certain fractions of thermostable proteins from barley, are capable of maintaining conditions motility, mitochondrial membrane potential and cell viability. However, the use of thermostable proteins fractions, not have a significant effect on protection of sperm cells Atlantic salmon in cryopreservation, still according to the results would be advisable to cryopreserve protein without them (control negative). Author acknowledges the support of FONDECYT 1151315 (Jorge Farías). FONDECYT 1151173 (León Bravo). CONICYT Doctorate grants (Stefania Short).

EFFECT OF VEGETARIAN FEEDING ON ANTHROPOMETRIC PARAMETERS AND SPERM QUALITY IN THE STUDENTS OF THE UNIVERSITY OF ANTOFAGASTA

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Vegetarianism is a food option that totally or partially excludes food of animal origin in your diet. In recent years there has been an increase in both Chile and the world by the option of totally restricting this type of food. It is known that people who adopt this type of diet are subject to possible deficiencies of micro and macro nutrients, altering the corporal functions, including those of the reproductive system.
The objective of this work was to study the effect of vegetarian feeding on the anthropometric parameters and sperm quality in the students of the University of Antofagasta. For this, we worked with a population of 110 students, of which 40 students are vegetarians and 70 students are non-vegetarians. In these, anthropometric measures were taken and a survey of healthy habits, eating patterns and in the case of men, were also carried out an analysis of sperm quality.
According to the results obtained, men and women who adopt a vegetarian diet tend to have a lower body mass index. In addition, vegetarian men have a higher percentage of high and very high fat mass. Regarding waist circumference, the results show that, in both men and women, the type of diet does not influence this parameter. Regarding sperm quality, the results show that, although both populations have adequate sperm parameters, Vegetarian diet exerts some kind of negative influence on sperm morphology, since in vegetarian men 93.2% of morphological alterations are observed versus 13% of abnormal spermatozoa in non-vegetarian men, these results suggest possible problems of subfertility. Fondecyt 1130341

CHARACTERIZATION AND SHORT-TERM STORAGE OF THE PATAGONIAN BLENNY (*ELEGINOPS MACLOVINUS*) SPERM

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Patagonian blenny (*Eleginops maclovinus*; namely robalo) population has been decreasing due to overexploitation. Little is known about the reproduction behavior of robalo, and even less about its reproduction biology. Fish sperm of several other teleost are described in literature, but research regarding fish sperm from native species from Chile is only beginning. Understanding the ability for Patagonian blenny sperm to sustain short-term storage is a key step to carry out artificial propagation of this species. In this aspect, sperm function markers (membrane and DNA integrity; reactive oxygen species generation), cell respiration/mitochondrial-function markers (mitochondrial membrane potential; dynamics of the ATP content; oxygen consumption), and also the pH and osmolarity of the extracellular medium during cold storage could be highly helpful for designing an *in-vitro* management protocol for Patagonian blenny. Patagonian blenny spermatozoa structure and ultrastructure are consistent with spermatozoa from modern teleost with external fertilization, measuring ~40 µM length, a head of ~0.8 µM diameter, and containing 3 to 5 spherical mitochondria. Semen contains ~15×109 spz mL-1, an osmolarity of ~345 mOsm kg-1 and pH of ~7.5. Analyses of sperm function were done during 14-day cold storage, under diluted (1:1 with Cortland solution) and undiluted conditions. The use of Cortland solution do improve the storage time from 3 to 7 days approximately, allowing better gas exchange, preventing desiccation and keeping membrane integrity better. Factors that affect most the storage time are reactive oxygen species (ROS) generation and unwanted motility process activation during storage, produced probably by osmolarity and ion content differences.
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REPRODUCTIVE PARAMETERS OF ATLANTIC SALMON (*Salmo salar*) FROM RECIRCULATION CULTURE SYSTEMS

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Early exposure to diverse environmental influences is associated with the occurrence of larval and/or embryonic malformations in different species. Likewise, it has been observed that Atlantic salmon (*Salmo salar*) from recirculation (water reused) systems have increased their percentage of malformations compared to traditional systems. On the other hand, fertilization and optimal development can be affected by damage to the germinal line, changes in maturation, structure and function of gametes. Therefore, the aim of this study is to characterize Atlantic salmon spermatozoa from recirculation systems through the analysis of reproductive parameters. For this, semen of 3 broods was collected by stripping. Membrane integrity and mitochondrial membrane potential were evaluated by flow cytometry using the Sybr-14/PI and JC-1 probes, respectively. Motility was assessed using the Computer Assisted Sperm Analysis (CASA) system. Observing that 98.1% of the sperm had an intact membrane, 65.8% had a high mitochondrial membrane potential indicating good cell function, and 98.7% of total motility (progressive motility=64.2%, non-progressive motility=34.5%). In relation to their velocity, 49.5% were fast and 45% was media, noting that the media velocity of the lateral displacement of the head (μm) was 2.1 and 1.5, respectively. As for the kinetic parameters (μm/s) of fast spermatozoa, the curvilinear velocity was 136.2, the rectilinear velocity was 104.8 and the average velocity was 126.7. It can be concluded that the analyzed spermatozoa present the appropriate quality to perform an optimal fertilization, supporting the hypothesis that malformations are these systems would be inherited and not due to the quality of spermatozoa.
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SERTOLI CELL-MEDIATED DIFFERENTIATION OF BOVINE FETAL MESENCHYMAL STEM CELLS INTO GERM CELL LINEAGE IN AN *IN VITRO* CO-CULTURE SYSTEM

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Due to their abundant source and high differentiation potential, mesenchymal stem cells (MSC) may be suitable candidates for *in vitro* gamete derivation. Nevertheless, germ cell differentiation requires endocrine and auto/paracrine regulation in a specific environment, as well as direct cell-to-cell interactions provided by the somatic cells of the testis. Sertoli cells (SC) play an essential role by forming niches for germ cells providing essential factors for germ cell differentiation. The aim of the present study was to evaluate the effect of co-culture of SC on bovine fetal MSC (bfMSC) differentiation into germ cell lineage. Sertoli cells were isolated from bull testis and characterized by quantification of biomarker wilms tumor 1 (WT1) expression and androgen binding protein (ABP) mRNA levels using flow-cytometry (FC) and quantitative-PCR (Q-PCR) analyses. bfMSC were isolated from adipose tissue and were co-cultured with SC for 21 days. Bovine testis samples (positive controls), fibroblasts (negative controls), bfMSC-SC co-cultures, bfMSC and SC were analyzed for expression of housekeeping genes β-ACTIN and GAPDH, and male germ cell gene DAZL by Q-PCR. High levels of WT1 and ABP mRNA were quantified in bovine SC and testes samples; however, no transcripts of these genes were detected in bovine fibroblasts. Moreover, a high ($P<0.05$) proportion (85,9%) of SC were positive for WT1. DAZL mRNA levels were up-regulated at day 21 of differentiation in bfMSC-SC co-cultures compared to bfMSC and SC controls. Preliminary results suggest that co-culture with SC may be an efficient *in vitro* system for bfMSC differentiation into germ cell lineage.
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