XXIX

Annual Meeting

SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

Abstracts Book

September 5th to 8th, 2018
Hippocampus Resort & Club
Concón, Chile

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Fundada el 30 de Abril de 1987

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Sharing knowledge and technology in Reproduction and Development Research

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XXIX ANNUAL MEETING
SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

Wednesday 5th

14:00-15:30 hrs **REGISTRATION**

15:45-16:00 hrs **OPENING CEREMONY**
Alfonso Paredes
SCHRD President

16:00-17:00 hrs **CONFERENCE AWARD SCHRD 2017**
Gabriel Mingo. Pontificia Universidad Católica de Chile
Vasculogenic Mimicry: An alternative model of tumor irrigation

17:00-17:30 hrs **Coffee**

17:30-19:00 hrs **SYMPOSIUM 1**
CANCER IN THE REPRODUCTIVE GLANDS
Chair: Héctor R. Contreras

Lilian Jara. Universidad de Chile
Germline variations in the miRNAs miR-423, miR-27a, miR-182, and miR-1245a: potential as familial breast cancer susceptibility alleles and role in cell transformation.

Carmen Romero. Hospital Clínico Universidad de Chile
New therapies in epithelial ovarian cancer: role of microRNA-145

Héctor R. Contreras. Universidad de Chile
Transcriptional factors and castration-resistant prostate cancer

19:00-20:00 hrs **CONFERENCE 1**
Chair: Dolores Busso

Rafael Fissore. University of Massachusetts, Amherst
Getting to know the Ca^{2+} toolkit of mouse oocytes and eggs: a puzzle worth solving.

20:00 hrs **Cocktail**

Thursday 6th

9:00-11:00 hrs **ORAL PRESENTATIONS SESSION**
Chair: Fernanda Parborell and Omar Espinoza

Cortasa SA, Proietto S, Schmidt RA, Corso MC, Inserra PIF, Leopardo N, Vitullo AD, Dorfman VB and **Halperin J**.
Growth and steroidogenesis of accesory vs. primary corpora lutea in pregnant vizcachas (Lagostomus maximus)

Del Río JP, Hevia, G, Rioseco H, Vigil P.
Association between allergen-specific immunologic skin reactions and reactive hyperprolactinemia in women with ovulatory disfunction

Heber MF, Ferreira SR, Abruzzese GA, Vega M, Motta AB.
Effect of *in utero* androgen excess on ovarian insulin signaling. Role of metformin treatment.

López-Moncada F, Torres MJ, Castellón EA, Contreras HR.
SPARC induces epithelial-mesenchymal transition, enhancing migration and invasion, and is associated with high Gleason score in prostate cancer.

Moreno RD, Buñay J, Marconi M, Larriba E and Del Mazo J.
Combined proteomic and mirnome analyses of mouse testis exposed to a mixture of endocrine disruptors chemicals reveal altered toxicological pathways involved in male infertility.

Oubiña G, Pascuali N, Scotti L, La Spina F, Buffone M, Higuera J, Abramovich D, Parborell F.
Phototherapy in female reproductive medicine: effects on ovarian function and possible uses in oncofertility.

Pacheco AB, Masone D, Altamirano KN, Beaumelle B, **Belmonte SA**.
HIV-1 TAT protein disrupts human sperm acrosomal exocytosis by blocking phosphatidylinositol 4,5-bisphosphate availability.

Peralta OA and Torres CG.
In vitro approaches for derivation of male germ cells from bovine fetal mesenchymal stem cells

11:00-11:30 hrs **Coffee**

11:30-13:00 hrs **SYMPOSIUM 2**
NEUROENDOCRINE CHALLENGES: CHANGES IN THE OVARY IN PERIMENOPAUSE.
Chair: Myriam Laconi

Antonio Martínez. Centro de Medicina Reproductiva de Mendoza
Ovarian aging: paradigm and current challenge

Myriam Laconi. Universidad Juan Agustín Maza y Universidad de Mendoza
Neurosteroids and ovarian function: effects during menopause

13:00-14:30 hrs **Lunch**

14:30-15:30 hrs **CONFERENCE 2**
Chair: Ricardo Moreno

Patricia Cuasnicú. Instituto de Biología y Medicina Experimental (IBYME) CONICET. Buenos Aires.
Molecular mechanisms involved in gamete interaction: from mouse to human

15:30-17:00 hrs **SYMPOSIUM 3**
ENERGY METABOLISM AND SPERM PHYSIOLOGY
Chair: Jorge Farías

Juan Reyes. Pontificia Universidad Católica de Valparaíso
Can glucose be part of the controlling mechanisms of mammalian meiotic transition and/or spermiogenesis?

Claudia Treviño. Universidad Autónoma de México
Calcium oscillations: a mechanism preventing the acrosome reaction

Manuel Lee. Universidad de La Frontera
Effects of cryopreservation on PKA in spermatozoa of S. salar

17:00-17:30 hrs **Coffee**

17:30-18:30 hrs **CONFERENCE 3**
Chair: Óscar Peralta

Charles A. Easley. University of Georgia
Using an *in vitro* spermatogenesis platform to model male factor infertility and develop novel therapies

18:30-19:30 hrs **POSTER SESSION (Cheese and Wine)**
Chairs: Pedro Orihuela, Gareth Owen

Arias A, Aisemberg J, Moreno RD and Sobrevia L
Endocrine disruptors alter the expression of estrogen receptor β and endothelial nitric oxide synthase in human umbilical vein endothelial cells

Cabrera-Cruz H, Plaza-Parrochia F, Oróstica L, Romero C and Vega M.
The insulin-sensitizer effect of myo-inositol in endometrial cells subjected to a polycystic ovary syndrome environment

Contreras MJ, Treulén F, Arias ME, Silva M, Felmer R.
Effect of the addition of antioxidants to the freezing medium on stallion sperm physiology

Coronado-Posada N, Olivero-Verbel J, and Moreno RD.
Effects of the preconceptional exposure to bisphenol and susceptibility to inflammation in a murine model induced with LPS-gain.

Díaz R, Dumorné K, Short S, Lee-Estevez M, Ulloa-Rodríguez P, Valdebenito I and Farías JG.
Effects of cold storage and cryopreservation on ultrastructure of atlantic salmon (*salmo salar*) spermatozoa

Dorfman VB, Cortasa SA, Schmidt AR, Proietto S, Corso MC, Inserra PIF, Di Giorgio NP, Vitullo AD, Halperin J.
The regulation of GnRh by prolactin is associated to corpora lutea activity during pregnancy in the South American plains vizcacha, *lagostomus maximus.*

Fuenzalida B, Cantin C, Carvajal L, Pasten V, Contreras S and Leiva A.
Human maternal supraphysiological hypercholesterolemia alter the traffic of cholesterol in placental trophoblast cells

Fuentes G, Ramírez MA and Sobrevía L.
Insulin reduces intracellular ph in human umbilical vein endothelial cells from gestational diabetes mellitus.

Henríquez S, Kohen P, Collins F, Saunders P and Devoto L.
Modulatory effects of Estrogens Metabolites (EM) on angiogenesis in endometrial stromal cells from women with or without endometriosis.

Hernández A, Vega M, Romero C.
Overexpresion of mir-23b on the proliferation and levels of c-Myc in epithelial ovarian cancer cell line.

Hevia G, Del Río JP, Rioseco H and Vigil P.
Association between a viral skin disease and reactive hyperprolactinemia in women with ovulatory disfunction.

Indo S, Torres MJ, Pérez G, Castellón EA and Contreras HR.
Generating an in vitro model of castration-resistant prostate cancer.

Jiménez D, García C and Contreras HR.
Effect of okadaic acid and its analogues on the viability of colon cell lines

Muñoz E, R. Felmer, M. Arias
Edition of the CSN2 (β-casein) gene in bovine fetal fibroblasts using CRISPR/CAS9 technology.

Pastén V, Hormazábal N, Cantin C, Fuenzalida B, Contreras-Duarte S and Leiva A.
Cholesterol efflux and antioxidant capacity of neonatal HDL from pregnancies with maternal hypercholesterolemia.

Peña S, Vargas C, Rubio M and Paredes AH.
Effect of Kisspeptin on the expression of Leukaemia Inhibitory Factor in rat ovaries during the subfertility period.

Pérez G, Torres MJ, Castellón EA, Contreras HR
Cancer stem cell phenotype inducction by the transcription factor ZEB1 in human prostate cancer cell line DU145.

Quiroz A, Fujiko S, Rigotti A and Busso D.
Maternal exposure to a western diet increases the incidence of neural tube closure defects in SR-B1-deficient mouse embryos.

Reuquén P, Curotto C and Orihuela PA.
Vaginocervical stimulation and intrauterine insemination of sperm cells differentially regulate the expression of Catehcol-*O*-Methyltransferase (COMT) and Tumor Necrosis Factor-alpha (TNF-alfa) receptors in the rat oviduct

Riquelme AN, Pérez G, Torres MJ, López-Moncada F, Contreras HR, Castellón EA.
DDX4 is expressed in samples of prostate cancer patients and is associated with pluripotency markers in cancer stem cells primary culture.

Riquelme R and Lara, H.
Effect of Stress in the Ovarian Cholinergic System, the Ovarian Function and the Fertility in the Rat.

Rodríguez-Ortiz R, Sanz A, Segura ML, Lara-Martínez R, Jiménez-García LF and Rodríguez-Gómez Y.
Ovogenesis and spermatogenesis of the banana frog *osteopilus septentrionalis* (*anura: hylidae*)

Saavedra F, Villarreal M, Molina P, Santander N, Rigotti A and Busso D.
Influence of vitamin E intake and plasma levels on the risk for developmental abnormalities in SR-b1 receptor KO mice.

Salvatierra R, Garrido MP, Hurtado I, Vega M, Romero C.
Effect of COX-2/Prostaglandin E2 on c-Myc, Survivin and VEGF protein levels in epithelial ovarian cancer cells.

Short SE, Bravo LA, Díaz R, Lee-Estevez M, Zepeda AB and Farías JG
Apoplastic extracts with antifreeze activity of *Dschampsia Antarctica* as a cryoprotectant of *salmo salar* spermatozoa.

Squicciarini V, Riquelme R, Wilsterman K, Bentley GE and Lara HE.
Role of RFP-3 in the development of cold stress-induced polycystic ovary phenotype in rats

Torres-Pinto I, Hernández A, Vallejos C, Garrido MP, Vega M, Romero C.
Overexpression of miR-145 decreases c-Myc protein levels and inhibits proliferation in an epithelial ovarian cell cancer line.

Torres MJ, Maturana M, Lefian A, López-Moncada F, Castellón EA, Tapia J, Llanos P and Contreras HR.
ET_AR is associated with expression of steroidogenic enzymes in prostate cancer cell lines.

Vallejos C, Garrido MP, Vega M, Romero C.
Effect of Metformin and Nerve Growth Factor (NGF) on mir-145 and mir-23B levels and the regulation of c-Myc and Vascular Endothelial Growth Factor (VEGF) levels on ephitelial ovarian cancer cells.

Vaquer CC, Pacheco Guiñazú AB, De Blas GA, Suhaiman L, **Belmonte SA.**
Ceramide promotes intracellular calcium increase in human sperm and triggers the exocytosis of the acrosome.

Zepeda AB, Miranda I, Valdebenito I, Farías JG and Moreno RD

Treatment of Atlantic Salmon Broodstock (*Salmo salar*) with GnRH affects the quality of their offspring.

Friday 7th

9:00-10:00 hrs **NEW MEMBERS SESSION**
Chair: Enrique Castellón

Contreras-Duarte S, Carvajal L, Garchitorena M, Subiabre M, Fuenzalida B, Cantin C, Farias M, Sobrevia L and Leiva A.
Gestational diabetes mellitus treatment and its relevance in glycemia: what is about lipid control and feto-placental endothelial function?

Garrido MP, Salvatierra R, Vallejos C, Hernández A, Valenzuela M, Vega M, Quest A, Romero C.
Inhibition of NGF/TRKA by metformin blocks epithelial ovarian cancer growth and angiogenesis potential

Leiva A, Fuenzalida B, Cantin C, Carvajal L, Pastén V, Garchitorena MJ, Hormazábal N and Contreras-Duarte S.
Human maternal supraphysiological hypercholesterolemia impairs placental endothelial function, cholesterol traffic and functionality of neonataL HDL.

10:00-11:00 hrs **CONFERENCE 4**
Chair: Jorge Farías

Alberto Darszon Israel, Universidad Nacional Autónoma de México
The role of pH in the acrosome reaction in mammalian spermatozoa

11:00-11:30 hrs **Coffee**

11:30-13:00 hrs **SYMPOSIUM 4**
MECHANISMS OF FETAL REPROGRAMMING
Chairs: Margarita Vega and Manuel Maliqueo.

Alicia B. Motta. Universidad de Buenos Aires
Effect of prenatal hyperandrogenization on the endocrine and metabolic health of the offspring

Gonzalo Cruz. Universidad de Valparaíso
Metformin treatment during pregnancy prevents reproductive abnormalities in the offspring of obese mothers

Germán Ebensperger. Universidad de Chile
Effect of gestational hypoxia in neonatal and adult pulmonary circulation structure and function

13:00-14:30 hrs **Lunch**

14:30-15:30 hrs **CONFERENCE 5**
Chair: Hernán Lara

George Bentley, University of California at Berkeley
Gonadotropin-inhibitory hormone in the gonads

15:30-17:00 hrs **SYMPOSIUM 5**
FERTILIZATION AND OOCYTE ACTIVATION
Chair: Dolores Busso

Ingrid Carvacho. Universidad Católica del Maule
Calcium channels as key proteins during oocyte maturation and fertilization

Marcela Michaut. Instituto de Histología y Embriología de Mendoza Dr. Mario Burgos
Role of alfa-SNAP in female fertility: lessons from a spontaneous mutant mice

Dolores Busso. Pontificia Universidad Católica de Chile
Ovarian Cholesterol Efflux: ABC Transporters and Follicular Fluid HDL Regulate Cholesterol Content in Mouse Oocytes

17:00-17:30 hrs **Coffee**

17:30-18:30 hrs **CLOSING CONFERENCE**
Chair: Margarita Vega

María Teresa Ruiz. Universidad de Chile
Nuestro universo: desde el big bang hasta la vida

21:00-02:00 hrs **CLOSING CEREMONY**
SCHRD Award for best oral communication
New Members Announcement
SCHRD 2019-2020 Presidente presentation: Jorge Farías
Dinner

Saturday 8th

10:00-12:00 hrs **Members meeting**

SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

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SCHRD AWARD 2017 CONFERENCE

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

Mingo G[1,6], Valdivia A[1,6], Racordon D[1], Aldana V[1], Sandoval A[6], González A[3], Cuello MA[1], Nualart F[4], Sanchez B[5], Corvalán AH[1,6] & Owen GI[1,2,6].

[1]Faculties of Biological Sciences & Medicine, Pontificia Universidad Católica de Chile. [2]Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile. [3]School of Medicine, Universidad San Sebastian, Santiago, Chile, & Center for Ageing and Regeneration (CARE), Pontificia Universidad Católica de Chile. [4]Faculty of Sciences, Universidad de Concepcion, Chile. [5]Institute of Physics, Pontificia Universidad Católica de Chile, Santiago, Chile. [6]Advanced Center for Chronic Diseases (ACCDiS), Pontificia Universidad Católica de Chile.

Vasculogenic mimicry (VM) is an alternative perfusion pathway utilized by cancer cells that is independent of endothelial cells. Although controversy still surrounds the existence of an *in vitro* model, the clinical presence of VM correlates with poor patient survival. Herein, we establish an *in vitro* assay of VM that recreates the formation of functional hollow channels using cancer spheres and primary cultures derived from ovarian cancers. Fluorescence confocal microscopy, X-ray microtomography 3D reconstruction and dye microinjection conclusively confirm the existence of functional glycoprotein-rich *in vitro* lined hollow structures. We report that the process of VM is distinct to that of angiogenesis and demonstrate the role of the extracellular matrix in triggering the formation of tubular structures. Using chemical inhibitors, we elucidate the intercellular pathways required to permit this phenomenon. A better understanding of the mechanism of VM may bring to light new prognostic biomarkers and allow the design of future anti-cancer therapies.

This study was supported by FONDECYT 1180241, CONICYT PFB12/2007, CONICYT-FONDAP 15130011, IMII P09/016-F, and the PUC Proyecto interdisciplina II15058.

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CONFERENCES

THE FERTILIZATION CA^{2+} TOOLKIT: A PUZZLE WORTH SOLVING.

Fissore R.
Department of Veterinary & Animal Sciences, College of Natural Sciences. University of Massachusetts Amherst.

Ca^{2+} oscillations are required for the initiation of development in all mammals. PLCzeta is the sperm protein thought to induce these oscillations in eggs during fertilization, although recent genetic studies raise doubts whether it is the only factor involved. We will discuss possible species differences among mammals. There are also Ca^{2+} oscillations prior to fertilization, as oocytes prepare to initiate oocyte maturation. We will describe the mechanism underlying these oscillations and the bioenergetic role they play in GV oocytes. Lastly, we will identify and characterize the myriad plasma membrane Ca^{2+} channels that permit Ca^{2+} influx from the extracellular media to support the oscillations during maturation or fertilization. These data highlight the extraordinarily complex Ca^{2+} toolkit that mammalian oocytes and eggs posses and that allow them to integrate different sources of $^{Ca2+}$ and even signaling molecules from the sperm to deliver carefully organized Ca^{2+} responses. Identification of the Ca^{2+} toolkit components and characterization of their regulation and function promises to improve the reproductive success of human and animals.

MOLECULAR MECHANISMS INVOLVED IN GAMETE INTERACTION: FROM MICE TO HUMANS

Cuasnicú PS.
Institute of Biology and Experimental Medicine (IBYME-CONICET), Buenos Aires, Argentina

Fertilization is a key process involving a series of coordinated interactions between the male and female gametes. Our laboratory has been dedicated to underpin the molecular mechanisms involved in fertilization using the evolutionarily conserved CRISP family (Cystein-Rich Secretory Proteins) as model molecules. Epididymal CRISP1 associates with sperm during epididymal maturation and participates in both sperm-zona pellucida binding and gamete fusion through its interaction with egg-complementary sites in rodents and human. More recent results reveal that CRISP1 is also expressed by the cumulus cells that surround the egg and acts as a sperm chemoatractant during cumulus penetration through its ability to regulate CatSper, the principal sperm Ca(2+) channel essential for male fertility. In spite of its functional relevance, CRISP1 KO mice are fertile as are mice lacking CRISP4, another epididymal CRISP family member also involved in fertilization. Interestingly, recent evidence from our group shows that the simultaneous lack of CRISP1 and CRISP4 affects not only fertilization but also male fertility, indicating the existence of compensatory mechanisms between CRISP homologues to ensure reproductive success. Moreover, double KO males exhibit an immature epididymal epithelium, defective sperm and an inflammatory phenotype (i.e epididymo-orchitis) indicative of a disruption of the high immunotolerance of the epididymis. Together, these observations reveal, for the first time the relevance of CRISP for male fertility and suggest novel inmunoregulatory roles for these proteins within the epididymis. We believe these results contribute to a better mechanistic understanding of epididymal physiology and pathology with clear implications for human infertility and contraception.

DERIVATION OF FUNCTIONAL SPERMATIDS FROM RHESUS MACAQUE EMBRYONIC STEM CELLS

Easley IV CA [1,2,3], Gill B[1,2], Punyawai K[3], Khampang P[3], Symosko KM[1,2], Fowler KL[1,2], Cho IK [3*], Steves AN [5], and Chan AWS[3,4].

[1]Department of Environmental Health Science, College of Public Health; University of Georgia; [2]Regenerative Bioscience Center; University of Georgia; [3]Division of Neuropharmacology and Neurologic Diseases; Yerkes National Primate Research Center; [4]Department of Human Genetics; Emory University; [5]Genetics and Molecular Biology Program, Laney Graduate School; Emory University; Atlanta, Georgia, 30322; United States.

Since 1980, Assisted Reproductive Technology (ART) has gained worldwide acceptance and Intracytoplasmic Sperm Injection (ICSI) has since aided couples with severe male factor infertility to achieve pregnancies. However, these techniques rely on the production of male gametes (sperm or spermatids) to fertilize a partner's oocyte in vitro. For those patients unable to provide sperm or spermatid samples, no treatment options are available. However, in vitro gametogenesis from patient-specific stem cells represents one potential cure. Using GFP-labeled, rhesus non-human primate ESCs (nhpESCs), we show that nhpESCs can be differentiated into VASA+ germ cell lineages that include PLZF+ spermatogonia; HIWI+ secondary spermatocytes; and Acrosin+, Protamine 1+, and Transition Protein 1+ haploid spermatids. Haploid spermatids generated in vitro possess correct parent-of-origin imprints on 2 loci. Furthermore, haploid spermatids produced in vitro are functional. Haploid spermatids derived in vitro can participate in fertilization of an nhp/rhesus macaque oocytes and undergo DNA decondensation, pronucleus formation/apposition, cell division, and development to the blastocyst stage in vitro. We also demonstrate a 12% blastocyst success rate. Taken together, our model demonstrates for the first time that functional male gametes can be derived from primate pluripotent stem cells and represent a potential therapy for male factor infertility.

THE ROLE OF PH IN THE ACROSOME REACTION IN MAMMALIAN SPERMATOZOA"

Darszon Israel, Alberto

Universidad Nacional Autónoma de México

Mammalian sperm must undergo capacitation as preparations to undergo motility changes known as hyperactivation, become able to acrosome react and acquire the ability to fertilize. One of the initial events in this process is when sperm encounter a HCO_3^- elevation. This anion activates the atypical adenylyl cyclase Adcy10, increases intracellular cAMP and stimulates protein kinase A (PKA). In addition, an intracellular Ca^{2+} ($[Ca^{2+}]i$) increase is essential for sperm capacitation. Although clearly a crosstalk between cAMP-dependent pathways and Ca^{2+} plays an essential role in sperm capacitation, the connection between these signaling events is not well understood. Here, we present three different approaches to show that CatSper, the main characterized sperm Ca^{2+} channel, is up-regulated by a cAMP-dependent activation of PKA. First, HCO_3^- and 8-Br-cAMP induce an increase in $[Ca^{2+}]i$ which is blocked by PKI, a peptide inhibitor of PKA activity and by HC-056456, an inhibitor of CatSper. Second, $HC0_3^-$ increases the membrane depolarization induced by allowing monovalent cations to flow through CatSper channels and this response is inhibited by PKI and HC-056456. Third, electrophysiological recordings revealed that CatSper activity was up-regulated by HCO_3^-, 8-Br-cAMP and by direct introduction of cAMP through the patch-clamp pipette. Activation by HCO_3^- and 8-Br-cAMP was blocked by PKI or HC-056456. Electrophysiological recordings in sperm from CatSper KO mice confirmed CatSper involvement in these modes of activation. In sum, these data strongly suggest that PKA-dependent phosphorylation is involved in the regulation of $[Ca^{2+}]i$ homeostasis through activation of CatSper channel complexes. Acknowledgements to FONDECYT 1180387 (IP: Jorge Farías)

GONADOTROPIN-INHIBITORY HORMONE IN THE GONADS

Bentley G.
University of California at Berkeley

Communication across the first endocrine axes in protochordates and basal vertebrates likely relied on diffusion. Because diffusion is relatively slow, rapid responses to some environmental cues may have required further local control (e.g., local regulation of steroid hormone production by the gonads). Despite the evolution of much more efficient circulatory systems and complex nervous systems in vertebrates, production of many "neuro"transmitters has been identified outside of the hypothalamus, and in particular in the gonads. These neurotransmitters are known to regulate endocrine function locally in specific tissues. Our understanding of gonad-specific neuropeptide expression and its role coordinating physiological/behavioral responses of the whole organism remains limited. Here, I review regulation of gonadotropin-inhibitory hormone (GnIH) across the reproductive axis in birds and mammals and discuss context-dependent differences and similarities in GnIH production by the brain and gonads. The implications of differential regulation in the brain and gonads will be discussed in terms of GnIH's role as a regulator of reproductive activity in response to environmental cues, such as stress, social environment and season.

IN SEARCH OF OUR COSMIC ORIGINS

Ruiz, Maria Teresa
Departamento de Astronomía, Facultad de Ciencias Físicas y Matemáticas. Universidad de Chile.

After thousands of generations of human evolution, today we have the privilege of being part of the first one to know about its cosmic origins and the path that brought us here. The great advances in science and technology have been crucial to pursue the exploration of our universe, increasing our ability to "observe" it and helping us to better understand the origin of life.

In this talk we will travel in space-time to learn about what we now know about the universe, from the Big-Bang to the present. It is an evolution in increasing degrees of complexity, from fundamental particles to conscious life, like ours.

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SYMPOSIUM 1
CANCER IN THE REPRODUCTIVE GLANDS

GERMLINE VARIATIONS IN THE MIRNAS MIR-423, MIR-27A, MIR-182, AND MIR-1245A: POTENTIAL AS FAMILIAL BREAST CANCER SUSCEPTIBILITY ALLELES AND ROLE IN CELL TRANSFORMATION"

Jara L.
Human Genetics Program, Institute of Biomedical Sciences (ICBM), School of Medicine, University of Chile, Santiago, Chile

MicroRNA(s) (miRNAs) are a novel class of endogenous RNAs, capable of regulating gene expression. Single-nucleotide polymorphisms (SNPs) can alter miRNA expression. We evaluated the association of SNPs rs6505162 (pre-miR-423), rs895819 (pre-miR-27a), and rs4541843 (pre-miR-182) with familial breast cancer (BC) risk in a Chilean population. Results showed that: a) rs6505162-C increases risk in families with a strong history of BC; b) the G/G genotype at rs895819: A>G reduces risk in families with a moderate history of BC; and c) rs4541843-T increases risk. Given that TOX3 is a putative target of miR-182 and that we have previously found that TOX3 rs3803662-T increases BC risk, we evaluated the combined effect of both BC risk alleles. We found a dose-dependent and additive effect of the number of risk alleles. Using an *in vitro* model, we evaluated the effect of rs6505162 (miR-423-3p and miR-423-5p) and rs895819 (miR-27a-3p and miR-27a-5p) on expression levels and migration rates in the BC cell lines MCF-7 and MDA-MB-231. rs6505162-C and rs895819-G increased expression of both strands of mature miR-423 and miR-27a. rs6505162-C increased migration in MCF-7 and MDA-MB-123, and rs895819-G increased and reduced migration in MCF-7 and MDA-MB-231, respectively. Sequencing pre-miR-1245a in 96 BRCA1/2-negative cases demonstrated that rs60611793 corresponds to one adenine deletion, located 22 bp upstream from miR-1245a. This SNP was detected in 5 of 277 (1.8%) BRCA1/2-negative BC cases and 3 of 192 controls (1.5%). All cases who were carriers of rs60611793 had early-onset BC ($\bar{x}$=31.8 years). This SNP may be associated with early-onset BC.

Funded by Fondecyt N° 1150117 y U Redes URC-007/17

ROL OF MIR-145 AS ADJUVANT THERAPY IN EPITHELIAL OVARIAN CANCER (EOC)

Romero C.
Laboratorio de Endocrinología y Biología de la Reproducción, Hospital Clínico Universidad de Chile. Departamento de Obstetricia y Ginecología. Facultad de Medicina, Universidad de Chile

Content protected by patent

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TRANSCRIPTIONAL FACTORS AND CASTRATION-RESISTANT PROSTATE CANCER

Contreras HR.
Programa de Fisiología y Biofísica. Instituto de Ciencias Biomédicas. Facultad de Medicina. Universidad de Chile.

Prostate cancer (PCa) is the second male malignancy in the world. The transcription factor ZEB1 associated to epithelial to mesenchymal transition (EMT) program has been involved in the regulation of the androgen receptor expression, Testosterone and DHT can be synthesized through the steroidogenic pathway within the prostate in a altered way in PCa. Methods: qRT-PCR, Western blot and immunocytochemistry were used to determine the mRNA, protein levels and cellular localization of EMT markers and enzymes of the steroidogenic pathway in the DU145 cell line with ZEB1 silencing. The concentrations of testosterone and DHT in the culture medium of these cells were measured by ELISA. Results: ZEB1-silenced cells showed an increase in the concentration of testosterone and DHT in the cell culture medium, an increase in the AR expression and an alteration in steroidogenic pathway, specifically a decrease in StAR and 5α-Reductase 2 expressions and an increase in CYP17A1, 5α-Reductase 1, AKR1D1 and AKR1C2 expression. Conclusion: The ZEB1 silencing induces an increase in the production of testosterone and DHT, associated with an increase of the CYP17A1 and 5α-Reductase1 enzymes in DU145 cell line. These results constitute a new finding on the regulation of intratumoral androgen production in PCa.

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SYMPOSIUM 2
NEUROENDOCRINE CHALLENGES: CHANGES IN THE OVARY IN PERIMENOPAUSE.

OVARIAN AGING: NEW PARADIGMS AND CHALLENGES

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The ovarian aging is defined as the physiological decrease of the functional ovarian reserve associated with age and implies a decline of the female reproductive potential as well as the related cyclic endocrine activities. The age at which menopause occurs holds also a clinical and public health interest because it may be a marker of aging and health in women.
The availability of new biomarkers for evaluation of the ovarian reserve and follicular dynamics across the reproductive lifespan, allows an assessment of the effects of both environmental and lifestyle exposures, as well as other factors that could impact germ cells during periods of vulnerability throughout life. This has facilitated the early identification of individuals at increased risk of shortening and premature decline of their reproductive capacity.
On the other hand, recent publications reports on the possibility of generating artificial gametes by the manipulation of somatic cells or pluripotent stem cells, which may be used to restore the primordial follicles and thereby the oocyte pool. These therapeutic interventions in female reproductive ageing would produce a profound impact in the field of medically assisted reproduction techniques.
It is evident that we are facing new scientific and social developments that are creating a challenging scenario, which requires a multidisciplinary approach by those who involved in the use and application of this knowledge and techniques.

NEUROSTEROIDS AND OVARIAN FUNCTION: EFFECTS DURING MENOPAUSE.

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Menopause is a physiological stage; changes that occur include variations in the hypothalamic-pituitary-gonadal axis that affect female behaviour and ovarian physiology. Changes in the ovary during menopause are due to a depletion of the follicular population that leads to a suspension of ovarian cycle and loss of oocyte quality. The steroids side effects on the effector organs decrease. Neurosteroids have a crucial role in the potentiation or attenuation of peri-menopausal effects. At physiological concentrations, they can attenuate anxiety, improve depressive symptoms and improve sexual appetite, while at peri-menopausal concentrations they cause the opposite effects. Changes in mood during perimenopause could be related with changes in neurosteriods levels, specifically with Allopregnanolona (GABAa agonist). We demonstrated the positive Allopregnanolone action over the anxiety and memory in an experimental model. Clinical trials have shown that lifestyle and dietary can improve deleterious effects of menopause. In addition, modification of serotonin reuptake inhibitors are the most useful option for the treatment of hot flushes when sex hormones are to be avoided.
The neurosteroids can act directly on the CNS or, indirectly, by influencing the neurotransmitter systems (serotonin and DA) modulating and attenuating the negative effects of the oestrogen's fall. The physiological effects attributed to oestrogen could be a consequence of changes in the serotonin efficacy and distribution of its receptors, related to estrogen. Integrating data from endocrinology, molecular biology and neurosciences, it is proposed that the neurosteroids, through their action on serotonin, could moderate the negative effects of the fall of estrogen during menopause.

SYMPOSIUM 3
ENERGY METABOLISM AND SPERM PHYSIOLOGY

FLUORESCENT GLUCOSE ANALOG UPTAKE AND GLUT1/MCT1 DISTRIBUTION IN RAT SEMINIFEROUS TUBULES.

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Most of the evidences supporting the transport and metabolic role of Sertoli cells (SC) in SC-germ cell interaction derive from *in vitro* SC properties. Cultured SC take up glucose, glycolytically metabolize it and release lactate to the media. However, the nurturing metabolic role of glucose of *in situ* Sertoli cells or the passage of glucose to the adluminal compartment in seminiferous tubules (STs) have not being experimentally tested. The basis of this proposal comes from many sources: GLUT1 (and other GLUT isoforms) are expressed in isolated SC. Although GLUT isoforms have been reported in rodent STs, the location of these transporters on SC cannot be clearly established from the available reports. Rodent spermatogenic cells have functional GLUT transporters and take up and metabolize glucose. This evidence and the fact that a non-metabolizable glucose analog can cross the haemo-testicular barrier, strongly suggest that, in vivo, glucose is likely to reach the adluminal compartment through the SC, where it would be taken up by spermatogenic cells. If this process were to occur, it would have Ca^{2+}- mediated consequences for spermatogenic cell function. In this work, using confocal fluorescence microscopy and measuring the distribution of two fluorescent glucose analogs: 6-deoxy-NBD-glucose (6NBDG, non-metabolizable); and 2 deoxy NBD-glucose (2NBDG, phosphorylated by hexokinase), a mitochondrial probe (tetra methyl rhodamine), a lipophilic impermeant probe (FM 4-64), and inhibitors of glucose transport and hexokinase, we were able to show that 2NBDG, a glucose analog that can be phosphorylated by hexokinase, accumulates in SCs giving a pattern of distribution that coincides mainly with SC structure toward the lumen of STs, passing slowly to spermatogenic cells. Inhibition of hexokinase permits an increased passage of 2NBDG to spermatogenic cells. 6NBDG, a glucose analog that is not a substrate of hexokinase was taken up by SCs, but also showed uptake into spermatogenic cells, in agreement with the notion that SC hexokinase is effectively a bifurcation for glucose metabolism and passage to the adluminal compartment in STs. The distribution of GLUTs isoform 1 is in agreement with the functional uptake of glucose transport in ex vivo STs. MCT1 distribution agrees with monocarboxylate release toward the adluminal and basal compartments in STs.

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CALCIUM OSCILLATIONS: A MECHANISM TO PREVENT THE ACROSOME REACTION

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During transit through the female reproductive tract, sperm encounter environmental conditions that modulate various processes leading to fertilization. Intracellular Ca^{2+} and pH_i dynamics regulate sperm functions such as capacitation, motility and the acrosome reaction (AR). In this work, we determined that during human sperm capacitation there is a pH_i increase that differs in each sperm subcellular region (head, mid and principal piece) and that HCO_3^- is a key regulator of these pHi changes which in turn regulate sperm motility. Pharmacological experiments revealed that several molecular entities are involved in these pHi changes. We also explored the occurrence of the progesterone induced AR in single human spermatozoa capacitated under external pH (pH_e) conditions found in different regions of the female reproductive tract (pH_e = 6.5, 7.4 and 8.0). The highest percentage of AR induction occurred when sperm were capacitated at pH_e 7.4. Interestingly, at pH_e 6.5 a high percentage of cells exhibit Ca^{2+} oscillations, those cells did not undergo the AR. These oscillations involve extracellular and intracellular Ca^{2+} channels. Pharmacological inhibition of Ca^{2+} oscillations restores the ability of spermatozoa to undergo the AR when exposed to progesterone, even if capacitated at pH_e 6.5. Our findings suggest that Ca^{2+} oscillations may represent a mechanism to prevent untimely AR in human sperm. This work was supported by PAPIIT IN-203116 to CT and acknowledgements to FONDECYT 1180387 (IP: Jorge Farías)

EFFECTS OF CRYOPRESERVATION ON CYCLIC-AMP DEPENDENT PROTEIN KINASE (PKA) IN SPERMATOZOA OF *S. SALAR*.

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Sperm motility in external fertilization fish lasts for short time, being critical for aquaculture, where high reproductive efficiency is required; hence optimizing sperm motility is particularly relevant. However, gametes preservation techniques, e.g. cryopreservation, reduce motility percentage and duration. Very few studies have addressed cryodamage from energetic and cell signalling points of view. In this study, activities of cAMP-dependent protein kinase (PKA) and AMP-activated protein kinase (AMPK) were measure in fresh and cryopreserved spermatozoa of Atlantic salmon (*Salmo salar*); and their possible involvement in cryopreservation-induced reduction in sperm motility is discussed. PKA-mediated protein phosphorylation is involved in sperm motility activation in salmonids, possibly through phosphorylation of axoneme proteins, while AMPK is involved in cell energy regulation and sperm motility in mammals and poultry; and it has been shown that AMPK activation improves sperm function and antioxidant defenses after freezing-thawing process. Our results showed significant decrease in membrane integrity and motility in post-thawed spermatozoa compared to fresh samples, consistently with previous reports. Both PKA and AMPK kinase activities
didn't exhibited differences before and after motility activation, while displayed significant decrease after cryopreservation ($p<0.05$), suggesting implication with the reduction of sperm quality. However, no significant correlation between PKA and AMPK activities was found, and *in-silico* docking analysis showed AMPK activation directly by PKA is unlikely. No previous reports of this phenomena in fish was found, making these findings interesting and worthy of further study. Moreover, potential biotechnological applications may be developed based on this and further studies.
This work was supported FONDECYT 1151315 and 1180387 (JF), FONDECYT Post-doctoral project N°3160572 (RD) and CONICYT National Doctorate Scholarships No. 21150246 (MLE).

SYMPOSIUM 4
MECHANISMS OF FETAL REPROGRAMMING

EFFECT OF IN UTERUS ANDROGEN EXCESS IN HEPATIC INSULIN RESISTANCE. ROLE OF METFORMIN TREATMENT

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The liver is the organ which controls body energy metabolism. A common liver disease is the development of insulin resistance, a pathological condition in which cells are unable to normally respond to insulin. There is evidence that androgen in uterus affects insulin sensitivity in several tissues. These findings led us to investigate whether in uterus androgen excess develops hepatic insulin resistance and the role of metformin as treatment.
For these purposes, we used a prenatally hyperandrogenized (PH) rat model in which two phenotypes were obtained: a PH irregular ovulatory (PHiov) and a PH anovulatory (PHanov) phenotype. Both PH phenotypes displayed hepatic insulin resistance characterized by decreased mRNA expression of IR, IRS1 and IRS2. Metformin treatment reversed only that of IRS1. Hepatic insulin resistance correlated with decreased mRNA gene expression of the glucose transporter GLUT2 in both phenotypes, while metformin reversed this decrease only in the PHanov phenotype. Concomitantly, we found decreased gene expression of the transcriptional factors CHREBP, SREBP and PPARg in both phenotypes. Metformin reversed mRNA gene expression of CHREBP in PHanov and of PPARg in both phenotypes. All these alterations correlated with increased hepatic oxidative stress in the PHanov phenotype, which was reversed by metformin. Our results show, for the first time, that androgen excess in uterus promotes hepatic insulin resistance, affecting glucose transport and transcriptional factors that modulate glucose metabolism and lipogenesis. Metformin treatment reversed some of these alterations in a specific-phenotype manner. These findings remark the importance to study the development not only of systemic but also of peripheral insulin resistance.

EFFECT OF GESTATIONAL HYPOXIA IN STRUCTURAL AND FUNCTIONAL PROPERTIES OF PULMONARY CIRCULATION FROM NEONATAL AND ADULTHOOD PERIOD

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Gestational chronic hypoxia induces in the newborn multiples cardiopulmonary maladaptation during the transition perinatal. This process is developed inefficiently involving alterations in the vascular reactivity, and in properties and morphological features of neonatal pulmonary circulation leading to the called "neonatal pulmonary hypertension syndrome". Newborn with this syndrome develop a vasoconstrictor hyperreactivity with the increase of pulmonary arterial wall thickness if they remain in an environment with conditions of chronic hypoxia maintaining an elevated resistance of pulmonary vascular compared to the pulmonary circulation of the newborns at sea level. Whether chronic hypoxia during neonatal period can change or program long-term disorders in physiological and vasoactive parameters associated with molecular and morphological pulmonary alterations, during adulthood will be reveal in the present study. The newborns exposed to chronic hypoxia during gestation and moved to normoxia at sea level after birth modify the properties of pulmonary circulation and show clear differences with those that were

maintained during the whole pregnancy at sea level. Can these changes be an affect of the diminution of environmental oxygen bioavailability or the results of a programming during gestation?

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METFORMIN TREATMENT DURING PREGNANCY PREVENTS REPRODUCTIVE ABNORMALITIES IN THE OFFSPRING OF OBESE RATS

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Maternal obesity associates with metabolic and reproductive dysfunctions in offspring. Female offspring of obese rats have increased serum estradiol levels during early postnatal life, leading to early onset of puberty and polycystic ovary condition in adulthood. Using metformin during pregnancy and nursing to improve the metabolic status of obese mothers we attempted to prevent the sequence of events leading increase in postnatal serum estradiol levels in female offspring and, hence, mitigate reproductive dysfunction. We found that metformin (160-200 mg/Kg) prevented the increase in serum estradiol levels in female offspring of obese mothers, probably due a restoration of its hepatic metabolization by cytochrome P450 3A2. Metformin did not prevent advanced puberty, but we observed that antral follicles, follicular cysts, and multi-oocyte follicles returned to control values in the female offspring of obese mothers treated with metformin. An increase in norepinephrine and 3-methoxy-4-hydroxyphenylglycol in the ovaries was also observed, indicating increased sympathetic activity in female offspring induced by an obesogenic uterine environment. We found that this effect was prevented by metformin administration. Despite the beneficial effects of metformin on reproductive parameters, we found increase bodyweight and increase retroperitoneal fat in offspring of obese rats. We concluded that metformin administration to obese mothers during pregnancy and nursing partially prevents ovarian dysfunction in female offspring during adulthood but may exacerbate the metabolic dysfunctions.

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SYMPOSIUM 5
FERTILIZATION AND OOCYTE ACTIVATION

CALCIUM CHANNELS AS KEY PROTEINS DURING OOCYTE MATURATION AND FERTILIZATION

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The proper maturation of oocytes is essential for supporting fertilization and the early embryonic divisions. In the ovary, immature fully-grown oocytes are arrested in prophase I of meiosis I. These oocytes are not able to support fertilization. Acquiring fertilization competence, a process called "oocyte maturation", requires resumption of meiosis, including the remodeling of multiple signaling pathways and the reorganization of cellular organelles. Oocytes undergo maturation reaching metaphase II of Meiosis II ("egg") which is the stage required for fertilization. During the oocyte maturation and in the early events associated with fertilization, Ca^{2+} plays a critical role in modulating intracellular signaling pathways. Moreover, Ca^{2+} has been shown to be the universal activator of development at fertilization. Therefore, variations on the pattern of expression, distribution and function of Ca^{2+} channels during oocyte maturation and egg activation are critical to reproductive success. Ca^{2+} channels from the family of voltage gated Ca^{2+} channels have been shown to be expressed in eggs. Recently, two members of TRP channels family, a group of non-selective cationic channels, have been recorded in oocytes, eggs and early embryos. The function of all these Ca^{2+} channels and the interaction between them will determine Ca^{2+} influx during oocyte maturation and in early embryonic development. I will discuss recent progress related with the expression and the role of Ca^{2+} channels in mammalian oocyte physiology.

ROLE OF ALFA-SNAP IN FEMALE FERTILITY: LESSONS FROM A SPONTANEOUS MUTANT MOUSE

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In most mammals, the main mechanism that prevents polyspermy during fertilization is the cortical reaction. Cortical reaction is triggered by the sperm and consists in the secretion of the content of the cortical granules. The membranes of the cortical granules fuse with the plasma membrane of the oocyte. Several studies have suggested that this fusion of membranes is mediated by the SNAREs proteins. The aim of our work was to identify and characterize the alpha-SNAP protein in mouse oocytes and its implication in female fertility. In a first stage, we worked with oocytes from CF1 females. Results of RT-PCR, Western blot and immunofluorescence showed that alpha-SNAP is present in oocytes and it is localized mainly in the cortical region enriched in cortical granules. Through a functional assay that allows evaluating the function of the protein in the cortical reaction, we demonstrated that alpha-SNAP regulates the cortical reaction in mouse oocytes activated parthenogenetically. In a second stage, we analyzed the fertility of the hyh females (hydrocephalus with hop gait), which present a point mutation in the alpha-SNAP gene. In vitro fertilization assays (IVF) showed that the IVF rate was lower for hyh oocytes with a

significant increase in the polyspermy rate. The functional analysis of the cortical reaction showed that hyh oocytes have an altered secretion of their cortical granules. Our results reveal that alpha-SNAP plays a fundamental role in female fertility.

OVARIAN CHOLESTEROL EFFLUX: ABC TRANSPORTERS AND FOLLICULAR FLUID HDL REGULATE CHOLESTEROL CONTENT IN MOUSE OOCYTES

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Cholesterol excess can be toxic for cells, so cholesterol levels are tightly regulated. Reverse cholesterol transport involves cholesterol efflux from cells to high density lipoproteins (HDL) via cell surface ATP binding cassette (ABC) transporters, followed by liver cholesterol uptake via scavenger receptor class B type I (SR-B1) and excretion into bile. HDL have been linked to fertility because these dense particles are the only lipoproteins class detected in follicular fluid (FF) and HDL composition has been associated to oocyte and embryo quality. SR-B1 knock-out (KO) female mice are infertile due the presence of large, cholesterol rich circulating HDL. SR-B1 KO female become fertile after lowering plasma HDL cholesterol levels using probucol. Recent evidence showed that SR-B1 KO eggs have cholesterol excess associated to an abnormal rate of spontaneous meiotic activation and high lability. We recently showed that cholesterol accumulation in SR-B1 KO oocytes starts at the antral stage of follicular development. Cholesterol excess in eggs is prevented *in vivo* by probucol treatment of mice and *in vitro* by incubation of eggs with purified wild type HDL. We also described that ABC transporters are present in eggs and that, interestingly, ABCA1 KO mouse eggs exhibit excessive cholesterol content and high spontaneous activation rates, phenocopying SR-B1 KO eggs. In sum, our results suggest that defective efflux due to abnormal HDL may contribute to cholesterol accumulation in eggs from SR-B1 KO mice. We propose that cholesterol homeostasis maintained by FF HDL and ABC transporters may be relevant for female fertility.

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ORAL PRESENTATIONS SESSION

GROWTH AND STEROIDOGENESIS OF ACCESORY VS PRIMARY CORPORA LUTEA IN PREGNANT VIZCACHAS (*LAGOSTOMUS MAXIMUS*)[1]Cortasa SA, [1]Proietto S, [1]Schmidt RA, [1]Corso MC, [1]Inserra PIF, [1]Leopardo N, [1]Vitullo AD, [1]Dorfman VB and **[1]Halperin J.**
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Unlike the vast majority of mammals, pregnant vizcachas develop accessory corpora lutea [aCL] in the middle of the gestational cycle. Herein, we studied how the morphological structure of the primary corpora lutea [pCL] evolves during pregnancy, and how the recruitment and development of aCL at mid-term propitiate the conditions to complete the gestation successfully. For that, we compared cell morphology and analyzed semi-quantitatively the immunoexpression of steroidogenic enzymes (Star, 3β-HSD, Cyp19 and 20-αHSD) in pCL vs aCL, relating these results with serum progesterone levels. pCL from early-pregnancy showed luteal cells with eosinophylic cytoplasm, abundant secretory granules and a 3:1 ratio cytoplasm:nucleus. In addition, at this stage we detected Star, Cyp19, 3β-HSD and 20α-HSD luteal immunoexpressions and 3.56 ± 0.2 ng/mL of serum progesterone. At mid-pregnancy, pCLs became disorganized and smaller and the cytoplasm:nucleus ratio of luteal cells markedly decreased. These regressing pCLs showed less 3β-HSD but more Star reactivity than active pCL whereas 20α-HSD remained unchanged. On the other hand, the recruited and formed aCLs showed luteal cells morphologically and steroidogenically similar to that of the active pCL. The known nadir of progesterone occurring at mid-pregnancy of vizcachas would be compensated by this new set of aCL, since at this stage the circulating hormone increased to 8.82 ± 0.9 ng/mL. The morphological and steroidogenic differences between aCLs and regressing pCLs observed in the ovaries of mid-pregnant vizcachas are compatible with an active role of aCL in supplementing the levels of progesterone to ensure embryonic viability until the end of pregnancy.

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ASSOCIATION BETWEEN ALLERGEN-SPECIFIC IMMUNOLOGIC SKIN REACTIONS AND REACTIVE HYPERPROLACTINEMIA IN WOMEN WITH OVULATORY DISFUNCTION

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Prolactin (PRL) acts as an immunomodulatory factor, and hyperprolactinemia has been associated to certain immune disturbances, including dermatological conditions, though its clinical significance and role in hypersensitivity reactions is unclear. Studies have suggested that normoprolactinemic women with ovulatory dysfunction who have a PRL hyperresponse to thyrotropin releasing hormone (TRH) stimulation test,are patients with a sporadic or "reactive" hyperprolactinemia, and that they respond to treatment with dopamine agonists. The purpose of this study was to evaluate whether a reactive hyperprolactinemia reflects an increase in dermatological inmune disorders, clinically assessed by allergic contact dermatitis (ACD) caused by nickel. An analytical cross-sectional study was carried out on reproductive age women who consulted for ovulatory dysfunction. As part of the study, a TRH test was performed. Secondarily, a clinical assessment for ACD caused by nickel was carried out. Of 145 women who consulted for ovulatory dysfunction, the median age was 25 years. 68.9% (100) had an altered TRH test and 31.1% (45) had a normal response. Of those with reactive hyperprolactinemia, 50% (50) presented ACD. In patients with a normal TRH test, 22.22% (10) were diagnosed with the disease. The Odds Ratio for ACD in cases of reactive hyperprolactinemia was 3.5 (95% CI 1.57-7.83). Our results show that patients who consult for ovulatory dysfunction and have reactive hyperprolactinemia are 3.5 times more likely to present this allergic reaction. These data suggest that PRL has a role not only in ovulatory function but also upon immune-mediated phenomena.

EFFECT OF *IN UTERO* ANDROGEN EXCESS ON OVARIAN INSULIN SIGNALING. ROLE OF METFORMIN TREATMENT.

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Insulin resistance is the decreased ability of insulin to mediate metabolic actions. Insulin controls ovulation and oocyte quality. Alterations on ovarian insulin signaling pathway, that inhibits glucose uptake, could compromise ovarian physiology. We aimed to investigate the effects of *in utero* androgen excess on ovarian insulin signaling pathway and evaluate the effect of metformin treatment. Pregnant rats were hyperandrogenized with 1mg of testosterone during days 16-19 of gestation; at adulthood prenatally hyperandrogenized (PH) female offspring, presented 2 phenotypes: oligo-ovulatory (PHiov) and anovulatory (PHanov), a third group without treatment was the control group. Half of each group was treated orally from day 70-90 of life with 50 mg/kg of metformin. After metformin treatment estral cyclycity was recovered in PHiov and partially in PHanov. Both PH groups presented systemic insulin resistance, that was reverted by metformin treatment. Protein expression of IR, IRS-1/2 and Glut4 were decreased in both PH groups. In the PHiov group metformin restored expression of all the mediators, whereas in the PHanov group only IR and IRS-1/2. IRS-1 phosphorylation was measured in tyrosine residues which activate the pathway and in serine residues which impairs insulin action. PHiov group presented high IRS-1 phosphorylation on tyrosine and serine residues. In contrast, PHanov showed augmented serine phosphorylation and low tyrosine phosphorylation. Metformin treatment lowered serine phosphorylation only in PHanov group. Our results suggest that PHanov group has a defective insulin action that's partially restored with metformin. The PHiov group has less severe alterations and metformin treatment results more effective in this phenotype.

SPARC INDUCES EPITHELIAL-MESENCHYMAL TRANSITION, ENHANCING MIGRATION AND INVASION, AND IS ASSOCIATED WITH HIGH GLEASON SCORE IN PROSTATE CANCER.

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Secreted Protein Acidic and Rich in Cysteine (SPARC) is a matricellular protein highly expressed in bone tissue, that act as a chemoattractant factor promoting the arrival of prostate cancer (PCa) cells to the bone marrow. However, the contribution of tumor SPARC during the early stages of tumor progression is still unclear. We propose that SPARC from tumor cells induces a mesenchymal phenotype, increasing migration and invasion of PCa cells.
Immunohistochemistry evaluation of SPARC in Tissue Micro Arrays containing prostate samples of low, intermediate and high Gleason Score (GS), and Benign Prostatic Hyperplasia was carried out. To determine the effect of SPARC on tumor cells, knock down and ectopic expression of SPARC were performed in PCa cell lines PC3 and LNCaP, respectively. In these cells, EMT markers and transcription factors were assessed by immunofluorescence, immunoblot and RT-qPCR. In addition, proliferation, wound healing and transwell assays were conducted.
SPARC is expressed in PCa primary tumors, associated to higher GS. Ectopic expression of SPARC induces Epithelial-Mesenchymal Transition (EMT), decreasing E-cadherin and Cytokeratin 18 and increasing N-cadherin and Vimentin in LNCaP cells. Moreover, SPARC induces the expression of EMT regulatory transcription factors Snail, Slug and Zeb1. Additionally, SPARC knockdown in PC3 cells decreases migration and invasion without modifying cell proliferation. Our results indicate that SPARC might facilitate tumor progression, through modification of cellular phenotype in cancer cells, and might play an important role in PCa aggressiveness.
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COMBINED PROTEOMIC AND MIRNOME ANALYSES OF MOUSE TESTIS EXPOSED TO A MIXTURE OF ENDOCRINE DISRUPTORS CHEMICALS REVEAL ALTERED TOXICOLOGICAL PATHWAYS INVOLVED IN MALE INFERTILITY.

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The increase of male idiopathic infertility has been associated with daily exposure of endocrine disruptors chemicals (EDCs) such as phthalates and alkylphenols. The aim of this work was to relate changes in the protein pattern expression with the posttranscriptional control of transcripts encoding proteins by miRNAs in testis of male mice exposed to mixture of EDCs. We combined proteomic and miRNome analyses of mouse testis chronically exposed to a low-doses of a mixture of 0.3 mg/kg-bw/day of bis (2-ethylhexyl) phthalate, dibutyl phthalate, benzyl butyl phthalate and 0.05 mg/kg-bw/day 4-nonylphenol and 4-tert-octylphenol, administrated in the drinking water from conception until adulthood. We analyzed fertility and global changes in the patterns of testis proteome by 2D-electrophoresis and mass spectrometry (MALDI-TOF), along with bioinformatic analyses of miRNA implicated in the control of deregulated proteins and their association with published data in human infertile patients. Results showed that exposed mice with a reduce fertility were associated with changes in the expression of 18 proteins (10 up-regulated, 8 down-regulated), their bioinformatic showed that most of them (89%) were involved in cell death. Furthermore, we found a group of 23 miRNAs/isomiRs (down-regulated) correlated with 6 up-regulated targets proteins (DIABLO, PGAM1, RTRAF, EIF4E, IVD and CNDP2) and we found that some of these miRNAs/proteins deregulations were reported in human testis with spermatogenic failures and subfertility or infertility. Overall, we suggest that the exposure to EDCs potentially leads to reproductive problems in men by some mechanisms that implicate changes in the interactions miRNAs/proteins involved in cell death. This work was supported by FONDECYT (1150352) and CONICYT (21120505), Chile, and MINECO (BFU2013-42164-R), Spain

PHOTOTHERAPY IN FEMALE REPRODUCTIVE MEDICINE: EFFECTS ON OVARIAN FUNCTION AND POSSIBLE USES IN ONCOFERTILITY.

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It is known that LLLT has beneficial effects on several processes including wound healing, pain and inflammation. LLLT modulates biological functions, including cell proliferation, apoptosis and angiogenesis, which in the ovary are intimately related to fertility. Premature Ovarian Failure (POF) is characterized by the disappearance of ovarian follicles in young women, which may be caused by chemotherapy. Current treatments for POF, mainly hormone therapies, are not completely effective. The present study proposes photobiomodulation as a strategy to protect the ovary in cancer patients undergoing chemotherapy. The objectives were: to evaluate the *in vivo* effect of LLLT on ovarian function **a)** in adult mice, and **b)** in POF model induced by chemotherapy. For objective ***a***, LLLT (100 and 200 J/cm^2) was applied to F1 mice (BalbC x C57 / BL6) (8 weeks). For objective **b)**, POF model was induced with cyclophosphamide (CTX, 75mg/weight) in F1 mice and LLLT (200 J/cm^2) was applied. **a)** *Adult mice:* LLLT (200 J/cm2) increased the % of primary and preantral follicles, whilst decreasing the percentage of corpora lutea compared to control ovaries ($p<0.05$). LLLT reduced the P4 concentration and the ovarian apoptosis ($p<0.01$). LLLT decreased the ovarian endothelial cell area and increased the periendothelial cell area ($p<0.05$). Additionally, LLLT enhanced oocyte quality. **b)** *POF model:* LLLT restored % primordial follicles to control values and decreased the % of atretic follicles ($p<0.05$). Therefore, the biomodulator effect of LLLT regulates follicular dynamics in physiological conditions while preserving ovarian reserve and decreasing follicular atresia in the POF model.

HIV-1 TAT PROTEIN DISRUPTS HUMAN SPERM ACROSOMAL EXOCYTOSIS BY BLOCKING PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE AVAILABILITY

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The acrosome reaction (AR) is a regulated calcium-dependent exocytosis necessary for fertilization. The HIV-1 transactivating protein (Tat) is released by infected cells and extracellular Tat enters uninfected cells by endocytosis inducing toxic effects. The aim of this work was to determine if HIV-1 Tat was able to enter a non-endocytic cell like the sperm thereby affecting gamete function. We incubated spermatozoa with recombinant wild type Tat (WT-Tat). WB and IFI assays demonstrated that, at physiological concentrations, Tat penetrates sperm membranes. To elucidate the mechanisms involved in Tat internalization we challenged sperm with Tat mutants. The W11 residue is required in this process. Exocytosis experiments demonstrated that WT-Tat inhibited progesterone (Pg)-induced AR. Tat binds phosphatidylinositol 4,5-bisphosphate (PIP_2) with high affinity and our group has shown that PIP_2 plays a key role in the sperm exocytic cascade. Additionally, we tested a Tat mutant unable to bind PIP_2. This mutant did not affect sperm exocytosis. We rescued Tat-induced inhibition of secretion by adding PIP_2. This suggests that Tat is sequestering PIP_2. We assumed that Pg-induced AR inhibition will occur due to lack of IP_3 synthesis. To test this argument, we resorted to the agonist of IP_3 receptors, adenophostin that rescued Pg-induced exocytosis after Tat inhibition. This result was confirmed by calcium measurements. These findings suggest that Tat requires the W11 residue to penetrate the plasma membrane and once inside the sperm a strong interaction with PIP_2 abolishes the AR. Our findings may contribute to elucidate unsolved issues concerning male subfertility in HIV patients.

IN VITRO APPROACHES FOR DERIVATION OF MALE GERM CELLS FROM BOVINE FETAL MESENCHYMAL STEM CELLS

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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 at deriving male germ cells (GC) from bovine fetal MSC (bfMSC) using two *in vitro* approaches based on the effect of exogenous bioactive factors (BMP4, RA and TGFβ1) or coculture with Sertoli cells (SC). First, bfMSC from bone marrow (BM-MSC) were exposed to differentiation media consisting in DMEM plus 10% FBS and supplemented with TGFβ1 (1, 10 or 100 ng/mL), BMP4 (10, 50 or 100 ng/mL) or retinoic acid (RA) (0.01, 0.1 or 1 μM). Second, the effect of SC interaction with BM-MSC or adipose tissue MSC (AT-MSC) was analyzed. In both approaches, the expression of pluripotent, GC and male GC markers was determined in bfMSC during 21 days. Exposure to RA was more effective in increasing ($p<0.05$) expression of DAZL and regulating expression of OCT4 and mRNA levels of NANOG. Cocultures of SC with BM-MSC achieved higher expression of Oct4 and Nanog compared to monocultures of SC or BM-MSC. Moreover, the proportion of cells positive for Dazl were higher in cocultures of SC with AT-MSC. Changes in gene expression profiles during exposure to bioactive factors or coculture with SC suggest that bfMSC have the potential to progress into early stages of GC differentiation.
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SOCIEDAD CHILENA DE REPRODUCCIÓN Y DESARROLLO

Fundada el 30 de Abril de 1987

NEW MEMBERS SESSION

GESTATIONAL DIABETES MELLITUS TREATMENT AND ITS RELEVANCE IN GLYCEMIA: WHAT IS ABOUT LIPID CONTROL AND FETO-PLACENTAL ENDOTHELIAL FUNCTION?

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Gestational diabetes mellitus (GDM) associates with hyperglycemia and in some cases with dyslipidemia. Its treatment focuses in glycemic control and scarce information refers to dyslipidemia-treatment or placental vascular dysfunction seen in this pathology. The goal is to evaluate the effect of GDM-treatment on lipid-levels and the effect of it in the umbilical vein reactivity. GDM (n=185) and control (N-C,n=23) pregnant women were classified as non-obese (N-GDM, N-C) or obese (O-GDM). Treatments were diet and/or metformin and/or insulin. At trimester 3 (T3) umbilical cord and maternal blood samples were collected.Triglyceride (TG), total-cholesterol (TC), low (LDL) and high (HDL) density lipoproteins were determined enzymatically. Vascular reactivity was assayed in umbilical vein rings, measuring maximum relaxation (R_{max}) in response to CGRP. N-GDM and O-GDM presented altered oral glucose tolerance test compared to N-C, other maternal and neonatal clinical variables were similar. During T3, TC-levels were diminished in O-GDM respect to N-C, due to decreased LDL and HDL-levels. O-GDM women treated with insulin showed higher TC due to augmented LDL-levels respect to N-GDM, the opposite was observed when O-GDM were treated with diet/metformin. Maximum relaxation in response to CGRP was impaired in N/O-GDM respect to N-C, although glycemia was controlled and independent of the treatment followed. Additionally, N-C with TC≥280mg/dL showed lower R_{max} compared to N-C with TC<280mg/dL. In N-GDM with TC≥280mg/dL R_{max} was lower than N-GDM with TC<280mg/dL. In conclusion, lipid levels are differentially regulated by maternal obesity and GDM-treatments. This phenomenon could be relevant for the fetoplacental vascular function altered in GDM.

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INHIBITION OF NGF/TRKA BY METFORMIN BLOCKS EPITHELIAL OVARIAN CANCER GROWTH AND ANGIOGENESIS POTENTIAL

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HUMAN MATERNAL SUPRAPHYSIOLOGICAL HYPERCHOLESTEROLEMIA IMPAIRS PLACENTAL ENDOTHELIAL FUNCTION, CHOLESTEROL TRAFFIC AND FUNCTIONALITY OF NEONATAL HDL.

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Maternal physiological hypercholesterolemia (MPH) results from requirements of the growing fetus. In some pregnant women total cholesterol (TC) increase over a physiological level (i.e, maternal supraphysiological hypercholesterolemia, MSPH). MSPH associates with atherosclerosis in human fetal vasculature. With the aim of determine the effect of MSPH in the placental endothelial function, cholesterol traffic and the functionality of neonatal HDL, lipid profile was determined in pregnant women from the Hospital UC-CHRISTUS (n=325). Women were classified as MPH or MSPH according to TC levels (TC ≤ or >280 mg/dL, respectively). Maternal and neonatal blood as well as the placenta were obtained. MSPH was determined in a 32% of the woman and associated with reduced vascular dilation of umbilical and placental microvascular rings. In primary cultures of human umbilical vein endothelial cells and human placental microvascular endothelial cells from MSPH placentas was determined reduced L-arginine/NOS activity and increased arginases/urea activity. In primary cultures of trophoblast from MSPH placentas was determined reduced LDL and HDL uptake as well as increased cholesterol efflux and intracellular levels of free cholesterol. Neonatal lipids levels were comparable in MPH and MSPH; however, the protein composition and function of HDL was different. In HDL from MSPH neonates was determined reduced antioxidant capacity, reduced NOS activity induction and increased cholesterol efflux capacity. In conclusion MSPH is a maternal metabolic alteration associated with placental endothelial dysfunction, altered placental cholesterol traffic and dysfunctional neonatal HDL. These changes could contribute to the early beginning of atherosclerosis described in MSPH neonatal vasculature.

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POSTER SESSION

ENDOCRINE DISRUPTORS ALTER THE EXPRESSION OF ESTROGEN RECEPTOR β AND ENDOTHELIAL NITRIC OXIDE SYNTHASE IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Intrauterine growth restriction (IUGR) is described as poor fetal growth during gestation. Endocrine disruptors (ED) alter the endogenous hormonal balance and signaling pathways mediating estrogen receptor β (ERβ) and endothelial nitric oxide synthase (eNOS) activation. We determined whether a mixture of ED alters the expression of ERβ and eNOS in human umbilical vein endothelial cells (HUVECs). HUVECs from normal pregnancies (n = 3) (Clinical Hospital UC-CHRISTUS, Chile) were cultured in sera (20%)-supplemented M199 (passage 3, 37°C, 5% O_2, 5% CO_2) with (24 hours) or without a mixture of di(2-ethylhexyl) phthalate (3.4 nmol/L), dibutyl phthalate (18 nmol/L), benzyl butyl phthalate (BBP, 2.6 nmol/L), 4-nonylphenol (1.4 µmol/L), 4-tert-octylphenol (400 µmol/L). eNOS and ERβ protein expression was evaluated by western blot and immunocytochemistry. The ED mixture caused a reduction in the total (~20%) and cytosolic (~32%) ERβ protein abundance but increased the ERβ nuclear/cytosolic ratio (~2 fold). The ED mixture also increased the total eNOS protein abundance (~11 fold), reduced (~31%) its phosphorylation at Thr^{495}, and did not alter its phosphorylation at Ser^{1177}. A preliminary assay in HUVECs from IUGR showed that the ED mixture caused an increase in the total eNOS protein abundance compared with normal pregnancies. These results suggest that ED could modulate the function of HUVECs by altering eNOS and ERβ protein expression.This research was supported by FONDECYT (1150377, 1150352) and VRI-UC PhD fellowship (AA).

THE INSULIN-SENSITIZER EFFECT OF MYO-INOSITOL IN ENDOMETRIAL CELLS SUBJECTED TO A POLYCYSTIC OVARY SYNDROME ENVIRONMENT

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The Polycystic Ovary Syndrome (PCOS) is an endocrine-metabolic prevalent disorder in women, characterized by hyperandrogenism and insulin resistance. Since last decade, Myo-inositol (MYO), a cellular endogenous compound, has been studied as a new pharmaceutical insulin-sensitizer for PCOS-patients. MYO enters the cells through a specific Sodium-Myo-inositol transporter (SMIT-1), increasing glucose uptake; however, its molecular mechanisms are unknown. The aim of this study was to evaluate MYO insulin-sensitizer effect in endometrial cells in PCOS conditions.
For this, protein levels of SMIT-1 were analyzed by immunohistochemistry on endometrial tissue of four study groups: Normal-weight (N-W), Obese (OB), OB-Insulin Resistant (OB-IR), OB-PCOS-IR women. Additionally, in an *in-vitro* model of human endometrial stromal cells was assessed MYO effect under hyperandrogenic and hyperinsulinic conditions (HA/HI). Protein levels of SMIT-1, GLUT4 and phosphorylation rate of AS160 were evaluated (WB and/or immunocytochemistry).
Tissue results show a diminution of SMIT-1 protein-level in OB-PCOS-IR endometria *vs* other study groups (*p<0,0001);* similarly, in stromal compartment, lower SMIT-1 levels were obtained (*p<0,0001 vs OB*). The *in-vitro* data indicate a negative effect of testosterone on SMIT-1 levels (*p<0,01 vs Basal),* being reverted by MYO-treatment (*p<0,05);* AS160 phosphorylation rate diminished with HA/HI and no differences were obtained when MYO was added to cell-culture. Under HA/HI, lower GLUT4 protein-levels were found (14%), restored to basal levels with MYO-treatment (*p<0,001).* Consequently, PCOS condition affect negatively SMIT-1 levels in endometrial tissue, similarly to that observed in HA/HI stromal cells. These results suggest that the glucidic homeostasis is positively affected by MYO-treatment as an insulin-sensitizer in PCOS endometria.
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EFFECT OF THE ADDITION OF ANTIOXIDANTS TO THE FREEZING MEDIUM ON STALLION SPERM PHYSIOLOGY

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Cryopreservation of stallion sperm has not yet achieved the efficiency observed in other mammalian species. Cryopreservation induces a decrease in plasma membrane integrity and sperm motility and increases DNA damage which finally leads to the cell death. In the clinical practice, this effect is visualized with a low fertility of the mare. Among the factors contributing to these damages are the oxidative stresses, since antioxidant enzymes found in greater percentage in the seminal fluid are removed during the cryopreservation process, contributing to a greater susceptibility of the spermatozoa to oxidative damage. Therefore, in the present study we sought to determine the efficiency of three antioxidants specific for reactive oxygen species found in cryopreserved stallion sperm, through the measurement of sperm quality variables associated with oxidative stress during cryopreservation. Sperm samples from 3 Chilote breed stallions were collected and immediately cryopreserved using Equiplus Freeze 1 step® freezing medium with the addition of the following antioxidants i) superoxide dismutase mimic (MnTBAP), ii) N-Acetyl-Cysteine (NAC), and iii) 5, 10, 15, 20-tetrakis (4'-sulfonate-phenyl) porphyrinate iron (FeTPPS). Plasma membrane integrity (SYBR/PI), mitochondrial membrane potential (TMRM/Sytox), lipid peroxidation (BODIPY/PI), and reactive oxygen species (DHE/SYTOX), were evaluated at 0 and 4

hours post thawed by a Becton Dickinson flow cytometer. Results show that NAC is detrimental for spermatozoa, because it does not show good viability and also has a high percentage of ROS. FeTPPS increased the viability of sperm but increased the peroxidative damage. However, addition of MnTBAP significantly decreased ROS production and lipid peroxidation and this effect was maintained during the incubation period. Future experiments will see to evaluate the effect of antioxidants on sperm motility, DNA fragmentation, and pellucid zone binding.
Acknowledgment: FONDECYT 1160467 and CONICYT 21181068

EFFECTS OF THE PRECONCEPTIONAL EXPOSURE TO BISPHENOL AND SUSCEPTIBILITY TO INFLAMMATION IN A MURINE MODEL INDUCED WITH LPS-GAIN

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Endocrine Disruptors (EDs) generate great concern within the scientific community due to its impact on human health and ecosystems. Bisphenol A (BPA) is one of the elements most found frequently in the environment, with evidence of adverse effects. The study of preconceptional exposure proposes an important way to analyze whether the lifestyle and habits of adults in their reproductive age may affect reproductive health and the inflammatory response in their offspring. We propose that preconceptional exposure to BPA in mice increases the susceptibility to the inflammatory response in the progeny treated with Lipopolysaccharide / Galactosamine (LPS-Gain). In the present investigation we evaluate the paternal BPA exposure effects on biomarkers of liver injury in the offspring with an LPS-Gain-induced hepatitis in a mouse model. Adult male mice received by intraperitoneal injection a dose of 50 mg/ kg-body weight per day for 7 days, then they were crossed with virgin females, and the offspring (males/females) were treated in adulthood with the LPS-GaIN mixture. Our data show that the offspring of exposed parents to BPA are more sensitive to damage than the offspring of control group, especially females, with death record 6-hours after treatment ends. In addition, ALT levels and histological changes in liver tissue of exposed group were significantly higher than that in control group after 96 hours BPA treatment ends. These findings suggested that paternal exposure to BPA can result in a susceptibility to inflammatory process and liver damage in their offspring. FONDECYT 1110778 and 1150352 (RDM). Scholarship Alliance of the Pacific 2018-2, Colciencias Scholar Call 647-2014 and 727-2015.

EFFECTS OF COLD STORAGE AND CRYOPRESERVATION ON ULTRASTRUCTURE OF ATLANTIC SALMON (*Salmo salar*) SPERMATOZOA

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The structural integrity of spermatozoa is very important for the processes of fertilization and embryo development. In aquaculture, cold storage and cryopreservation has been used for reproductive practices, however these biotechnological tools generate a reduction of the fertilization capacity of sperm due to membrane damage. The aim of this study was to investigate the effects of cold storage and cryopreservation on ultrastructure of Atlantic salmon spermatozoa. Semen from three broodstock (n=3) was collected by stripping, an aliquot of each ejaculate was stored at 4ºC for 7 days and another aliquot was frozen in liquid nitrogen using a commercial extender supplemented with dimethylsulfoxide (DMSO), glucose and BSA 2%. The ultrastructure of spermatozoa was examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). We observed morphological changes induced by the cold storage and cryopreservation in the spermatozoa. At day-7 of cold storage, some spermatozoa (20%) showed breakage of plasma membrane of the head; weakening of the midpiece, resulting in the detachment of the flagellum and mitochondria; and breakage of the plasma membrane of the flagellum. In post-thaw spermatozoa (67%), severe damage in the plasma membrane and mitochondria was visible. Severe morphological distortion, including the deformed nuclei, bursting of the plasma membrane, vesicles distributed over the head and flagellum surface, vacuolated nuclei and detached or missing mitochondria and tails. Therefore, our results suggested that the fine structures of the *Salmo salar* spermatozoa are extremely susceptible to cold storage and cryopreservation having deleterious effects on ultrastructural morphology of spermatozoa, especially on membrane.
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THE REGULATION OF GnRH BY PROLACTIN IS ASSOCIATED TO CORPORA LUTEA ACTIVITY DURING PREGNANCY IN THE SOUTH AMERICAN PLAINS VIZCACHA, *Lagostomus maximus*

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Prolactin (PRL) promotes luteal steroidogenesis in rodents and inhibits hypothalamic–hypophyseal–ovaric axis during pregnancy. The South American plains vizcacha shows GnRH increase at mid-pregnancy with ovulation and corpora lutea (CL) formation. Herein, we investigated PRL modulation of GnRH expression and delivery and its relation to the CL development during gestation. We designed 2 experiments: 1) study of total release of GnRH from hypothalamic explants (eHT) of non-pregnant vizcachas cultured for 6hs in media supplemented with either hyperprolactinemic or control serum (n=5), 2) analysis of GnRH pulsatility in eHT of females treated for 7 days with Sulpiride (20mg/Kg, n=5) or with saline solution (controls). In addition, hypothalamic immunoexpression of GnRH, dopamine receptor (DR2), tyrosin hydroxylase (TH) and PRL receptor (PRLR) was studied in early-, mid- and term-gestating vizcachas (n=5/group). The mass of GnRH released from eHT incubated for 6hs with hyperprolactinemic serum was significantly increased related to controls ($p<0.05$). However, GnRH pulsatility was lower in hyperprolactinemic eHT compared to controls ($p<0.05$). The

expression of GnRH, TH, DR2 and PRLR was studied in the hypothalamus of pregnant vizcachas to associate it with the dual effect of PRL on GnRH. TH-immunoreactive neurons were localized in the neighboring of GnRH-cells, whereas co-localization of GnRH with DR2, and of TH with PRLR, progressively increased during pregnancy. These results suggest that the increase of GnRH at mid-gestation is induced by the concomitant increase of PRL which is also involved in CL formation, whereas the sustained exposition to PRL up to the term-gestation inhibits GnRH pulsatility.Funding: Fundación Científica Felipe Fiorellino, PIP110/14, PICT1281/2014.

HUMAN MATERNAL SUPRAPHYSIOLOGICAL HYPERCHOLESTEROLEMIA ALTER THE TRAFFIC OF CHOLESTEROL IN PLACENTAL TROPHOBLAST CELLS

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Placental trophoblasts modulate cholesterol traffic (uptake via SR-BI and LDLR and efflux via SR-BI, ABCA1 and ABCG1) from maternal to fetal circulation. Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal development, but maternal supraphysiological hypercholesterolemia (MSPH) leads to endothelial dysfunction and atherosclerosis in fetal vessels. Our aim was to determine the effect of MSPH on the traffic of cholesterol in human trophoblast. Pregnant women from Hospital Clínico UC-CHRISTUS with total cholesterol (TC) ≤280 or >280 mg/dL were considered as MPH (n=6) or MSPH (n=6). Trophoblasts were isolated from placentas by trypsin/DNAse digestions. Cholesterol uptake of fluorescent labeled LDL was reduced in MSPH cells (8.2 ± 1 vs 16.9 ± 4 ng/mg) without changes in HDL uptake. LDLR protein abundance was lower in MSPH cells (0.15 ± 0.1 vs 1.5 ± 0.2 arbitrary units) and SR-BI was unchanged. Cholesterol efflux determined in cells pre-incubated with [^{3}H] cholesterol was higher in MSPH cells compared to MPH (33 ± 0.02 vs 17 ± 0.05%) which was associated with reduced ABCG1 (0.3 ± 0.1 vs 1.6 ± 0.4) without changes in ABCA1 or SR-BI. Levels of TC were comparable between MPH and MSPH cells, cholesterol ester was reduced (3.7 ± 1 vs 6.1 ± 1), non-esterified cholesterol was increased (5.8 ± 0.4 vs 4.3 ± 1) and no changes in protein abundance of HMGCR was observed in MSPH respect to MPH cells. In conclusion MSPH alter the traffic of cholesterol in placental trophoblast cells without changes in the key enzyme for cholesterol synthesis.
Support: FONDECYT 1150344, 3180442. Conicyt and Faculty of Medicine PUC–PhD fellowships.

INSULIN REDUCES INTRACELLULAR pH IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS FROM GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) and the Na^+/H^+ exchanger 1 (NHE1)-mediated alkaline intracellular pH (pHi) increase nitric oxide generation in human umbilical vein endothelial cells (HUVECs). We assayed whether the basal pHi is alkaline in HUVECs from GDM and the potential role of NHE1 in this phenomenon. HUVECs from normal (n = 5) or GDM (n = 5) pregnancies (Clinical Hospital UC-CHRISTUS, Chile) were cultured in sera (20%)-supplemented M199 (passage 3, 37°C, 5% O_2, 5% CO_2) with (8 hours) or without insulin (1 nmol/L). The pHi was measured in cells loaded with 2,7-bicarboxyethyl-5,6-carboxyfluorescein acetoxymethyl ester (12 mmol/L) and exposed to NH_4Cl (20 mmol/L). Basal and pHi recovery (*dpHi/dt*) were estimated (10 seconds) after exposure to 5 mmol/L 5-N,N-hexamethylene amiloride (HMA, Na^+/H^+ exchangers general inhibitor), 0.1 mmol/L zoniporide (Zn, NHE1 inhibitor), 0.1 mmol/L concanamycin A (V-ATPases inhibitor), 10 mmol/L Schering (H^+/K^+-ATPase inhibitor). HUVECs from GDM show higher basal pHi than normal pregnancies (pHi 7.64 ± 0.11 versus 7.07 ± 0.03, respectively) (mean ± S.E.M., unpaired ANOVA, $P<0.05$) in absence of inhibitors. Insulin partially reversed GDM-alkaline pHi (7.32 ± 0.08). Without insulin, the *dpHi/dt* in GDM (1.68 ± 0.25 $x10^{-3}$ pH units/second) was higher than normal pregnancies (0.49 ± 0.03 $x10^{-3}$ pH units/second). Insulin increased the *dphi/dt* in normal pregnancies (1.9 ± 0.2 fold) but blocked the GDM effect. Zn and HMA blocked the GDM-increased *dpHi/dt* with or without insulin. In conclusion, HUVECs from GDM shows alkaline pHi due to higher NHE1 activity, a phenomenon restored by insulin. Acknowledgements: FONDECYT 1150377 (Chile) and SEMILLERO 5309 Universidad de Antofagasta (Chile). GF holds an MSc fellowship from the Universidad de Antofagasta (Chile).

MODULATORY EFFECTS OF ESTROGENS METABOLITES (EM) ON ANGIOGENESIS IN ENDOMETRIAL STROMAL CELLS FROM WOMEN WITH OR WITHOUT ENDOMETRIOSIS.

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The pathogenesis of endometriosis has not yet been elucidated, because it is a complex multifactorial disease. Women with endometriosis have an optimal peritoneal environment for the adhesion of endometrial cells to the peritoneal wall. Several studies have confirmed neovascularization around endometriotic lesions and there is a correlation between severity of endometriosis and the angiogenic activity of the peritoneal fluid. Also, it has been reported the suppression of growth and angiogenesis of endometriotic lesions by 2ME2 in model mice suggesting reduced 2ME2 production in ectopic endometria. 2-ME2 and 2-OHE2 have antiangiogenic, antiproliferative and proapoptotic effects, that may be beneficial for the treatment of endometriosis. Therefore the goal is to determine the modulatory effects of Estrogens Metabolites (EM) on angiogenesis in endometrial stromal cells (ESC) from women with or without endometriosis. The ESC are isolated from the endometrial biopsy, and cultured for 24 hours with 2ME2 and 2OHE2 at different concentration. The angiogenic potential (AP) was evaluated by *in vitro* angiogenesis assays of conditioned media of ESC culture. The VEGF levels were quantified in the conditioned media by ELISA and cell viability was detected by MTS assay. The endometriotic

ESC has increased their AP compared to the normal ESC in basal condition. The EM reduce the AP and VEGF levels in normal and endometriotic ESC, but this effect is increased on endometriotic ESC. The cell viability was not affect by EM. Finally, these results show the antiangiogenic effect of both EM on ESC; this effect is increased on endometriotic cells, suggesting the possible therapeutic effect of these EM on endometriosis.
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OVEREXPRESION OF MIR-23B ON THE PROLIFERATION AND LEVELS OF C-MYC IN EPITHELIAL OVARIAN CANCER CELL LINE

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ASSOCIATION BETWEEN A VIRAL SKIN DISEASE AND REACTIVE HYPERPROLACTINEMIA IN WOMEN WITH OVULATORY DISFUNCTION

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Prolactin (PRL) acts as an immunomodulatory factor, and hyperprolactinemia has been associated to certain immune disturbances, though its clinical significance is unclear. It has been noted that some alterations in the immune system are associated with an increased prevalence of infectious skin diseases. Studies have suggested that normoprolactinemic women with ovulatory dysfunction who have a PRL hyperresponse to thyrotropin releasing hormone (TRH) stimulation test, are patients with sporadic or "reactive" hyperprolactinemia, and they respond to treatment with dopamine agonists. The purpose of this study was to evaluate whether a reactive hyperprolactinemia reflects an increase in dermatological infections, clinically assessed by oral herpes. An analytical cross-sectional study was carried out on reproductive age women who consulted for ovulatory dysfunction. As part of the study, a TRH test was performed. Secondarily, a clinical assessment for oral herpes was carried out. Of 145 women who consulted for ovulatory dysfunction, the median age was 25 years. 68.9% (100) had an altered TRH test and 31.1% (45) had a normal response. Of those with reactive hyperprolactinemia, 24% (24) presented oral herpes. In patients with a normal TRH test, 11.1% (5) were diagnosed with the disease. The Odds Ratio for oral herpes in cases of reactive hyperprolactinemia was 2.53 (95% CI 0.9-7.12).
Our results show that patients who consult for ovulatory dysfunction and have reactive hyperprolactinemia are 2.53 times more likely to present this cutaneous virosis. These data suggest an association between reactive hyperprolactinemia and viral skin diseases.

GENERATING AN IN VITRO MODEL OF CASTRATION-RESISTANT PROSTATE CANCER

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Prostate cancer (PC) is the second most common cancer worldwide and represents the fifth cause of death in men. While there are screening methods available for the early detection of this cancer, approximately 20% of the patients continue to present with advanced disease at the time of diagnosis to which androgen deprivation treatment (ADT) is applied. Patients who undergo ADT, after 18 to 24 months, progress to the stage castration-resistant prostate cancer (CRPC). Actually, there are target therapies to different molecular pathways that are involved in the progression of CRPC, but none has been effective in increasing the survival of these patients. This, added to the low availability of CRPC biological samples, makes it necessary to generate an *in vitro model* to evaluate different biomarkers and their changes in response to different therapies. In this work, the levels of expression of key molecules in the development of PC and the steroidogenic pathway, such as PSA, androgen receptor (AR), 5□-reductase 1, Snail and BRCA1/2 were evaluated by RT-qPCR in PC primary cultures grown in androgen-free conditions. In our model, a significant increase in mRNA levels was observed in all the molecules studied compared to controls with interesting variations in the cells subcultures. Similar results have been obtained in previous investigations in our laboratory, in other cell lines. This observations would contribute to establish our model as a reliable model in the CRPC study.
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EFFECT OF OKADAIC ACID AND ITS ANALOGUES ON THE VIABILITY OF COLON CELL LINES

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Okadaic acid (OA), dinophysistoxin-1 (DTX-1), and dinophysistoxin-2 DTX-2 are marine toxins responsible for a gastrointestinal syndrome called diarrheic shellfish poisoning (DSP). These toxins are Ser/Thr protein phosphatases inhibitors, mainly 2A phosphatases. Although, there is no registry of human deaths caused by DSP, it has been reported that OA and its analogues have tumor promotion and carcinogenic effects. There are studies that correlates colorectal cancer, and other digestive cancers, incidence with contaminated shellfish consumption.
The objective of this work was the study of viability, using MTT and PP2A inhibition by immunoprecipitation of this toxins, on three colorectal cell lines: a non tumoral cell line (CON841), a primary tumor cell line (SW480) and a metastatic cell line (SW620), to evaluate the role of these toxins on colorectal cancer progression.
The highest PP2A inhibition regarding control was DTX-1 for all the cell lines used, and the lowest was DTX-2. SW620 showed higher PP2A inhibition level than the other lines for all toxins. Viability results showed that CON841 was the most sensitive line to the three toxins (IC50 AO: 54.38 nM; IC50 DTX-1: 43.53 nM; IC50 DTX-2: 81.17 nM) and SW620 was the most resistant cell line. (IC50 AO: 137.8 nM; IC50 DTX-1: 192.9 nM; IC50 DTX-2: 202.9 nM).
Results suggest that tumoral cell lines are more exposed to noxious effects of toxins due to their greater resistance to cytotoxic effects of the toxins compared to a non tumoral cell line. We propose that marine toxins may accelerate tumor progression in tumoral cell lines.
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EDITION OF THE CSN2 (β-CASEIN) GENE IN BOVINE FETAL FIBROBLASTS USING CRISPR/CAS9 TECHNOLOGY

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Cow's milk is one of the most consumed drinks in the world. However, the presence of milk proteins can cause food allergies in a large part of the child population, a condition known as cow´s milk allergy (CMA). One of the most allergenic proteins described in cow´s milk is β-casein (CSN2). Allergies to this protein may persist throughout life.
Recent CRIPSR/Cas9 technological breakthrough has made animal genome editing feasible, being currently widely used in research to generate gene knockout or knock-in in a variety of cells and organisms. The aim of the present study was to knockout the CSN2 gene using CRISPR/Cas9 technology. For this, we designed 4 different gRNAs for a region of the CSN2 gene and cloned these fragments into the pSPgRNA vector. Then, bovine fetal fibroblasts were co-transfected using a complex of Lipofectamine with pSPgRNA and pST1374-Cas9 vectors. To determine CRISPR/Cas9-assisted gene targeting efficiency, the T7 endonuclease I assay (T7EI assay) was used on annealed PCR products from presumptive edited fibroblast genomes. Preliminary results showed that CSN2 gene was effectively edited by indels. These data represent the first attempts to knockout the β-casein gene in mammal's cells. Future studies are underway to confirm the type of genetic modification experienced by these cells and assess the efficiency of this technology in a model of bovine embryos produced *in vitro*.
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CHOLESTEROL EFFLUX AND ANTIOXIDANT CAPACITY OF NEONATAL HDL FROM PREGNANCIES WITH MATERNAL HYPERCHOLESTEROLEMIA

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Pregnancy associates with a physiological (MPH) or supraphysiological (MSPH) increase of maternal total cholesterol (TC), which is required for fetal development. MSPH is determined in pregnancies with maternal TC> 280 mg/dL. MSPH associates with endothelial dysfunction and atherosclerosis of the feto-placental vasculature and it is unknown whether the neonatal lipoproteins have a normal or altered function in this condition. With the aim of determine the cholesterol efflux and antioxidant capacity of neonatal high-density lipoproteins (nHDL), umbilical cord blood was obtained from MPH (N=8) and MSPH (N=8) neonates. nHDL were purified by gradient ultracentrifugation. The cholesterol efflux assays were performed in primary cultures of human umbilical vein endothelial cells (HUVEC) loaded for 24h with [^{3}H] cholesterol followed by incubation with nHDL from MPH or MSPH. The antioxidant capacity of nHDL was determined in HUVEC by reactive oxygen species (ROS) quantification using 2',7'-dichlorofluorescein diacetate, in the absence or presence of $CuSO_4$. The level of maternal CT was higher in MSPH than MPH (317 ± 29 and 225 ± 35 mg/dL, respectively). The neonatal CT was lower than the maternal one and comparable in both groups (62 ± 10 and 64 ± 11 mg/dL, respectively). The cholesterol efflux of nHDL from MSPH was higher (31 ± 6%) and the antioxidant capacity was lower (56 ± 10%) compared to nHDL from MPH. In conclusion, MSPH associates with changes in the efflux and antioxidant capacity of neonatal HDL, which could be related to the endothelial dysfunction and atherosclerosis described in the feto-placental vasculature in MSPH.
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EFFECT OF KISSPEPTIN ON THE EXPRESSION OF LEUKAEMIA INHIBITORY FACTOR IN RAT OVARIES DURING THE SUBFERTILITY PERIOD.

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Kisspeptidergic system participates in the regulating of follicular development in the rat ovary. Kisspeptin (Kiss) promotes the transition from primordial to primary follicles in rat, favoring the ovulatory process in primates. It seems to acts through, or in collaboration with leukemia inhibitory factor and its receptor system (LIF/LIFR) because LIF also promotes ovarian follicular development. We do not know if this mechanism is operative during aging and if exists a relationship between Kiss and LIF system. To test these possibilities, we studied the changes in LIF / LIFR system during the subfertile period and its regulation by the kisspeptidergic system. Sprague Dawley rats at 6 (fertile period) and 9 months old (subfertile period) were used. We measured LIF and LIFR mRNA (qRT-PCR) and protein levels by western-blot. To test for a local effect of kisspeptin, ovaries from rats during fertile and infertile age were incubated *in vitro* in the presence of Kisspeptin (100ng/ml). Each group of animals have n=5.
The results obtained show that LIFR mRNA increased during subfertile period. LIF mRNA and LIF protein levels decreased in the ovary during the same period. The presence of Kiss in an *in vitro* study, increased the intraovarian protein content of LIF from ovaries obtained from fertile rats but not during the subfertile period. The decreased action of LIF and their function as stimulators of follicular development found during aging, could be important to understand the accelerated loss of the follicular reserve that normally occurs during reproductive aging in women.

CANCER STEM CELL PHENOTYPE INDUCCTION BY THE TRANSCRIPTION FACTOR ZEB1 IN HUMAN PROSTATE CANCER CELL LINE DU145

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Cancer stem cells (CSCs) have the ability of extending the tumor cell population and give rise to other type of cancerous cells given its undifferentiated state. CSCs constitute an important part of the development and expansion of a tumor having a role in the development of metastasis and resistance to chemotherapy.Transcription factor ZEB1 is fundamental in the mesenchymal epithelial transition (EMT) and in the expression of pluripotency genes in cells with CSCs phenotype.
This work proposes that ZEB1 induces the phenotype of CSCs in a prostate cancer (PCa) line cell DU145. To test this hypothesis, we evaluated the expression of CSCs phenotype markers SOX2, KLF4, CD44 and CD133 through RT-PCR, western blot and immunofluorescence, in DU145 cell line, expressing and silencing ZEB1. The ability to form prostate-spheres by these cells also was evaluated, obtaining a new CSCs population and replicating the characteristics of a tumor.
A low expression of CSCs phenotype markers SOX2, CD44 and CD133 and a great ability to generate prostate-spheres were observed in cells silenced for ZEB1 compared to those that were not silenced. Similar results were obtained by western blot. KLF4 mRNA and protein increase in cells silenced for ZEB1 in comparison with not silenced control.
These results suggest that ZEB1 regulates CSCs phenotype which may contribute to the understanding of tumor heterogeneity and to the development of better diagnosis tools and clinical treatment.
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MATERNAL EXPOSURE TO A WESTERN DIET INCREASES THE INCIDENCE OF NEURAL TUBE CLOSURE DEFECTS IN SR-B1-DEFICIENT MOUSE EMBRYOS.

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Scavenger Receptor Class B Type I (SR-B1) transports lipids, i.e. cholesterol and vitamin E, from/to HDL lipoproteins. SR-B1 is present in extraembryonic membranes during mouse early development. SR-B1 deficient embryos exhibit neural tube defects (NTD), vitamin E deficiency and high levels of reactive oxygen species. These abnormalities are prevented by feeding dams a vitamin E-enriched diet, suggesting that oxidative damage may contribute to NTD in SR-B1 KO embryos. Here, we hypothesized that maternal intake of a high-fat/high-sugar (HFHS) diet, previously shown to increase overall oxidative stress and reduce vitamin E bioavailability, increases the risk for NTD in our model. SR-B1$^{+/-}$ dams were fed chow (n=8) or HFHS diet (n=16) (60% kcal from fat plus 42g/L sugar in water) during 8 weeks, mated with SR-B1$^{+/-}$ males and fed the same diets until E9.5 (day of neural tube closure completion), when embryos were retrieved. No differences were observed in maternal weight gain (3.6±0.2 vs. 4.5±0.6 g) or in the number of embryos/dam (7±0.8 vs. 7±0.3) from chow or HFHS conditions (Student t-test). The incidence of NTD increased with both SR-B1 deficiency and maternal HFHS exposure (**Table 1**, p=0.016, Spearman Rank test). NTD was associated to developmental delay in SR-B1$^{+/-}$ and SR-B1$^{-/-}$ embryos from the HFHS group (mean somite numbers 11.4±1 and 11.3±1.2, respectively vs. 19.4±1.5 in WT embryos ($p<0.05$, One-Way Anova, Dunn´s post-test). Further studies will be pursued to determine if both SR-B1-mediated embryonic vitamin E deficiency and high maternal oxidative status contribute to the risk of NTD and other abnormalities in this model.

Table 1.	SR-B1$^{+/+}$	SR-B1$^{+/-}$	SR-B1$^{-/-}$
Chow diet	0% (0/15)	9% (2/22)	46% (6/13)
HF/HS diet	9% (4/19)	33% (13/40)	57% (7/15)

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Vaginocervical stimulation and intrauterine insemination of sperm cells differentially regulate the expression of Catehcol-*O*-Methyltransferase (COMT) and Tumor Necrosis Factor-alpha (TNF-alpha) receptors in the rat oviduct

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Mating shut-down an estradiol (E_2) non-genomic pathway by inhibiting Catehcol-*O*-methyltransferase (COMT) activity and stimulating the Tumor Necrosis Factor-alpha (TNF-alpha signaling in the rat oviduct. Herein, the independent contribution of two components of mating as vaginocervical stimulation (VCS) and sperm cells were studied. Estrous rats were subjected to mechanical stimulation of the vagina and cervix or inseminated with 10-20 millions of spermatozoids and 12 h later oviducts were excised. mRNA levels of *Comt* and the TNF-alpha receptors *Tnfrsf1a* and *Tnfrsf1b* were determined by Real-time PCR, localization and expression of COMT, TNFRSF1A and TNFRSF1B proteins were analyzed by immunofluorescence and Western-blot. VCS did not decrease oviduct levels of the transcripts and proteins of COMT, TNFRSF1A or TNFRSF1B and not change their tissue localization in the oviduct, which are mainly localized in the epithelial cells. In contrast, presence of sperm cells in the genital tract decreased between 70% and 40% mRNA and protein levels of COMT, but did not change its localization. Furthermore, *Tnfrsf1a* and *Tnfrsf1b* increased between 60% and 50% respectively, although protein expression as well as their localization did not vary after insemination. We conclude that the presence of

spermatozoids in the genital tract, instead VCS, utilizes COMT and TNF-alpha signaling in order to shut-down the E_2 non-genomic pathway in the rat oviduct.
BASAL PB0807, DICYT021743OD_DAS.

DDX4 IS EXPRESSED IN SAMPLES OF PROSTATE CANCER PATIENTS AND IS ASSOCIATED WITH PLURIPOTENCY MARKERS IN CANCER STEM CELLS PRIMARY CULTURE.

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Primary tumors of prostate cancer (PCa) are characterized by high cell heterogeneity. Cancer Stem Cells (CSCs) are tumor cells capable of recapitulate original tumor with their different cell populations. It has been reported that germline cell marker DDX4 is expressed in different types of cancer and, as potential stemness supporting gene, could be expressed in CSCs of PCa. As high Gleason Score (GS) tumors have more undifferentiated cells, we propose that the expression of DDX4 would be higher in samples of high GS when compared to benign prostatic hyperplasia (BPH) samples, and in primary cultures of cells in non-adherent conditions (CSCs) compared with adherent cells. We analyzed DDX4 expression by immunohistochemistry in TMA (tissue microarray) from patients with different GS (Ethical Committee approval). Additionally, it was assessed by immunofluorescence the DDX4 protein levels as a germline marker, CD44 and CD133 as stem cells markers, SOX2 and KLF4 as pluripontency markers, E-cadherin as EMT marker, Androgen Receptor (AR) and PSA as differentiation markers in CSCs and adherent cultures of PCa. The expression of DDX4 is increased in PCa samples of high GS when compared to BPH. In primary cultures, protein levels of DDX4, CD44, CD133, SOX2, KLF4 and E-cadherin are increased in CSCs compared to adherent cells. Protein levels of PSA and AR are decreased in CSCs compared to adherent cells. These results indicate that DDX4 is associated with stemness genes and could play a role in the maintenance of stem cells in a CSC phenotype.
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EFFECT OF STRESS IN THE OVARIAN CHOLINERGIC SYSTEM, THE OVARIAN FUNCTION AND THE FERTILITY IN THE RAT

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The mammalian ovary control steroids biosynthesis and follicular development. Polycystic Ovarian Syndrome (PCOS), presents an aberrant follicular development, hyperandrogenism and in most of the cases infertility. An increase in sympathetic tone by chronic exposure to cold stress induces a phenotype similar to polycystic ovarian condition but no information exist about the impact in fertility. Cholinergic system activation increased follicular development and fertility on the rat. The purpose of this work was to determine the effects of cold stress on the cholinergic system and its relation with rat´s fertility. Female Sprague-Dawley rats were exposed to cold stress during 28 days (4°C, during 3 h/day) to activate the autonomic nerves. They were followed by another 28 days period without stress, to verify the nerve activation and its relation with cysts development and fertility in the rats. At the end of each period, we measured plasma levels of steroids (enzyme immunoassay). For follicular development, we did a morphometric analysis. To verify sympathetic and cholinergic system we measured NA and ACh. Fertility was determined by the capacity of the experimental rats to maintain a cyclic ovulatory performance and to get pregnant. Stress increased both NA and ACh ovary at 28 days. Stress decreased the number of corpora lutea. Cystic follicles increased at the end of the period of observation, with an increased testosterone and estradiol plasma levels. Regarding fertility, there was a constant decrease in the ovulatory performance verified by the irregular estrual cycling activity suggesting that all of the changes found in the ovary, triggered by stress, and closely correlated to ovary function.
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OVOGENESIS AND SPERMATOGENESIS OF THE BANANA FROG *OSTEOPILUS SEPTENTRIONALIS* (ANURA: HYLIDAE)

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Osteopilus septentrionalis, known as banana frog, is the only existing species in Cuba that represents the family Hylidae, of the order Anura. This species is the most successful adaptively with a primitive pattern of reproduction by the presence of larval stages. So far, no studies had been conducted on the gonadal morphology of this species based on a histological description. To study of its reproduction may help to explain its adaptive success and it would provide necessary evidence for the preparation and development of handling and conservation programs. The aim of this study was to describe the stages of the *Osteopilus septentrionalis* spermatogenesis and ovogenesis by microscopy techniques. The 30 males and 37 females were collected in Matanzas province, Cuba in the months of October 2013 to September 2014. The right gonads were fixed in paraformaldehyde at 4% and the lefts in glutaraldehyde at 2.5%. The obtained gonads were processed through classical histology methods. The samples were stained with Hematoxylin-Eosin (H-E) and contrasted using uranyl acetate and lead citrate for later observation in optical and electronic transmission microscopy, respectively. This species matches the pattern of reproduction described in the literature about anura. Females go through the development of previtellogenic, vitellogenic and postvitellogenic oocytes. Males have a spermatogenic development by cysts and the mature spermatozoid is formed by head and tail, without accessory structures. Spermatozoids present a subacrosomal space in their heads, which lacks a conical perforatorium, and in their tails have an axoneme with long arms of dynein and abundant mitochondria. Ovaries are pigmented, unlike testicles. We thank the Laboratory of Morphology of the Basic Area of the International Center for Neurological Restoration (CIREN) and the Cellular Nanobiology Laboratory of the Faculty of Sciences of the National Autonomous University of Mexico (UNAM) for the support received for the processing and analysis of the samples.

INFLUENCE OF VITAMIN E INTAKE AND PLASMA LEVELS ON THE RISK FOR DEVELOPMENTAL ABNORMALITIES IN SR-B1 RECEPTOR KO MICE

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SR-B1 transports lipids between cells and HDL lipoproteins. This receptor is present in extra-embryonic membranes during early pregnancy. SR-B1$^{-/-}$ embryos from SR-BI$^{+/-}$ intercrosses are vitamin E deficient and have 1:2 prevalence of neural tube defects (NTD), malformations that are completely prevented by postconceptional maternal vitamin E dietary supplementation. Here, we evaluated the link between vitamin E deficiency and NTD risk in SR-B1$^{-/-}$ embryos from SR-B1$^{-/-}$ intercrosses. SR-B1$^{-/-}$ females are infertile, but this condition can be reversed by administration of the lipid-lowering drug probucol. SR-B1$^{-/-}$ females were fed probucol-supplemented diet (0.5%) for two weeks, crossed with SR-B1$^{-/-}$ males and then fed control chow (**GROUP 1**, n=7) or vitamin E-enriched chow (2,000 IU α-tocopherol/kg) (**GROUP 2**, n=7) from conception until embryo dissection at E9.5. SRB1$^{-/-}$ embryos from SR-B1$^{-/-}$ probucol-treated females exhibited NTD in an identical proportion in both groups [24% (11/46), p=1 Fisher´s exact test]. Developmental delay (embryos at an earlier Thelier stage than expected) was also found in 50% of NTD embryos from groups 1&2. As α-tocopherol plasma lowering by probucol (shown by other groups and our

preliminary data) at conception may contribute to developmental delay and NTD in SR-B1$^{-/-}$ embryos, in **GROUP 3**, chow with vitamin E + probucol was supplied to SR-BI$^{-/-}$ females (n=4) 2 weeks before conception and until E9.5. Only 2/28 (7%) embryos of the expected developmental stage showed NTD in this group (p=0.1132, Fisher´s test vs. GROUP 1). These results suggest that both low maternal plasma vitamin E levels and deficient SR-B1-mediated embryonic uptake may affect developmental progression and neural tube formation.
*Both authors equally contributed to this work.
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EFFECT OF COX-2/PROSTAGLANDIN E2 ON C-MYC, SURVIVIN AND VEGF PROTEIN LEVELS IN EPITHELIAL OVARIAN CANCER CELLS.
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APOPLASTIC EXTRACTS WITH ANTIFREEZE ACTIVITY OF *DESCHAMPSIA ANTARCTICA* AS A CRYOPROTECTANT OF *SALMO SALAR* SPERMATOZOA
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Cryopreservation is an important tool for the protection of genetic resources in aquatic species with high commercial value that allows for the conservation of germ cells, such as with Atlantic salmon (*Salmo salar*). However, freezing may cause cell damage affecting the sperm quality. New procedures including antifreeze proteins (AFPs) seem to improve sperm quality after cryopreservation. AFPs have the ability to bind to ice crystals inhibiting their growth, and ice recrystallization (IRI) *in vitro*. *Deschampsia antarctica* is a freezing tolerant vascular plant species (LT_{50} -27°C) exhibiting apoplastic antifreeze activity. We hypothesize that AFPs from *D. antarctica* favor the sperm quality of cryopreserved *S. salar* spermatozoa. The aim of this work is to evaluate cryoprotection of apoplastic extracts with antifreeze activity from *D. antarctica* in *S. salar* spermatozoa. Cryopreservation of *S. salar* spermatozoa has been made with a standard freezing medium (C+) and different treatments of apoplastic extracts (0.08 mg/mL) of *D. antarctica* supplemented with 1.3M DMSO, 0.3M glucose and 2% w/v BSA cryoprotectants. Post-thawing plasma membrane integrity (PMI) by SYBR-14/PI and mitochondrial membrane potential (MMP) by JC-1 markers were assessed using flow cytometry. Thawed cells in presence of apoplastic extracts without BSA maintained PMI as well as C+. The percentage of cells thawed with apoplastic extracts and cryoprotectants showed higher MMP than C+. However, after 30 days the MMP decrease for all treatments. Apoplastic extracts from *D. antarctica* showed a cryoprotective effect in *S. salar* spermatozoa. These could act as a non-permeating cryoprotectant, replacing BSA in the standard freezing medium.
Author acknowledges the support of FONDECYT 1180385 (Jorge Farías). FONDECYT 1151173 (León Bravo). CONICYT Doctorate grants (Stefania Short).

ROLE OF RFRP-3 IN THE DEVELOPMENT OF COLD STRESS-INDUCED POLYCYSTIC OVARY PHENOTYPE IN RATS

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RFamide-related peptide (RFRP-3), is a regulator of GnRH secretion from the brain, but it can also act in human ovary to influence steroidogenesis. We aimed to study the putative local role of RFRP-3 in the ovary and its potential participation in the development of a polycystic ovary phenotype induced by chronic sympathetic stress (cold stress). We used adult Sprague-Dawley rats divided into control and stressed groups. In both groups we studied the effect of intraovarian exposure to RFRP-3 on follicular development and plasma ovarian steroid concentrations. We also tested the effect of RFRP-3 on ovarian steroid production in vitro. Chronic in vivo intraovarian exposure to RFRP-3 decreased basal testosterone concentrations and cold stress induced progesterone production by the ovary. In vitro, RFRP-3 significant decreased to half the hCG induced ovarian progesterone and testosterone secretion. Immunohistochemistry and mRNA expression analysis showed a three-fold times decrease in RFRP-3 and expression of its receptor in the ovary of stressed rats, a result which is in line with the increased testosterone levels found in stressed rats. In vivo application of RFRP-3 recovered the low levels of secondary and healthy antral follicles found in stressed rats. Taken together, our data indicate a previously unknown response of hypothalamic and ovarian RFRP-3 to chronic cold stress, influencing ovarian steroidogenesis and follicular dynamics. Thus, it is likely that RFRP-3 modulation in the ovary is a key component of development of the polycystic ovary phenotype.

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OVEREXPRESSION OF miR-145 DECREASES c-Myc PROTEIN LEVELS AND INHIBITS PROLIFERATION IN AN EPITHELIAL OVARIAN CELL CANCER LINE

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ET_AR IS ASSOCIATED WITH EXPRESSION OF STEROIDOGENIC ENZYMES IN PROSTATE CANCER CELL LINES

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Androgen deprivation is the first-line treatment for locally advanced and metastatic Prostate Cancer (**PCa**). However, PCa patients often develop androgen resistance conducting to a Castration-Resistant Prostate Cancer (**CRPC**). Evidence shows that tissue from a CRPC patient, show over-expression of androgen biosynthesis enzymes. Additionally, elevated concentrations of endothelin-1 (**ET-1**) have been reported in CRPC patients. ET-1 is synthesized by both epithelial prostatic and tumor cells, and interacts with endothelin A receptor (**ET_AR**). Several studies have shown an over-expression of ET_AR, in advanced PCa. Furthermore, cell lines expressing high levels of ET_AR, produces testosterone under the stimulation of ET-1. The aim of this work was evaluate the association between the expression of ET_AR and steroidogenic enzymes in PCa cells.
ET_AR expression was evaluated in androgen-independent PC3/DU145 and androgen-sensitive LNCaP cell lines of PCa by immunofluorescence and western blot. Also, mRNA of steroidogenic enzymes, ET_AR and Pre-pro-ET-1 was quantified by RT-qPCR in all PCa lines.
High Expression of ET_AR was detected in PC3 and DU145 cell lines. On the contrary, LNCaP cells express low levels. In addition, Pre-pro-ET-1 mRNA in androgen-independent cells were higher than in androgen-sensitive cells. Furthermore, mRNA levels of CyP11A1, CyP17A1, AKR1C1, AKR1C3, AKR1C3, AKR1D1 and SRD5A2 were higher in PC3 than in LNCaP cells.
These results showed different ET_AR expression depending on androgen sensitivity of cells . Additionally, the presence of ET_AR correlates with changes in the expression of steroidogenesis enzymes pathway, suggesting a role for ET_AR in the PCa progression.
Acknowledgment: Fondecyt 1151214 (**HC**) and 11150243 (**PL**), Scholarship CONICYT 21160703 (**MJT**) and 21160886 (**FLM**) and U Redes URC-007/17.

EFFECT OF METFORMIN AND NERVE GROWTH FACTOR (NGF) ON MIR-145 AND MIR-23B LEVELS AND THE REGULATION OF C-MYC AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) LEVELS ON EPHITELIAL OVARIAN CANCER CELLS

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CERAMIDE PROMOTES INTRACELLULAR CALCIUM INCREASE IN HUMAN SPERM AND TRIGGERS THE EXOCYTOSIS OF THE ACROSOME

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The acrosome is a membrane-limited granule that overlies the sperm nucleus. In response to physiological stimuli, sperm undergo a calcium-dependent exocytosis of this granule termed: acrosome reaction (AR). Ceramide´s role in exocytosis is not well defined, since it's been shown that in some cases it positively regulates membrane fusion while in others has the opposite effect. Also, is not well characterized if its effects on secretion are exerted by itself or by related metabolites. We evaluated the role of ceramide and its metabolites in the AR by using biochemical and exocytosis assays. WB and IFI analysis demonstrated the presence of enzymes of the sphingolipid metabolism in human sperm. Both, C6-ceramide (C6-Cer) treatment or endogenous ceramide increase induce AR in capacitated sperm in a percentage similar to progesterone. Human sperm loaded with Fluo3-AM, a calcium sensor, responded to C6-Cer with a transient increase in calcium concentration whose profile was similar to that elicited by progesterone. Furthermore, ceramide increase promotes calcium mobilization from internal stores (ryanodine receptors) however it requires extracellular calcium influx through SOCCs to accomplish acrosome release. The ceramide or progesterone-elicited AR was inhibited by NVP-231, a ceramide kinase blocker, indicating that both inducers require ceramide 1-phosphate (C1P) in the pathway leading to AR. Further, the inhibition was reverted by adding C1P. These findings led us to the conclusion that probably C1P is regulating AR after a ceramide increase. Here, we identified ceramide and C1P as novel molecules integrated in a signaling cascade leading to membrane fusion during acrosomal exocytosis.

TREATMENT OF ATLANTIC SALMON BROODSTOCK (*Salmo salar*) WITH GnRH AFFECTS THE QUALITY OF THEIR OFFSPRING

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The embryonic development can be affected by different endogenous and exogenous factors, where several investigations in mammals have shown negative effects in the offspring of parents exposed to an unfavorable environment. For this reason, it is hypothesized that the use of GnRH in culture of Atlantic salmon reproducers promotes offspring with deformations during their development For this study, four experimental groups were evaluated: 1) males and females without GnRH (MOG/FOG), 2) males and females with GnRH (MWG/FWG), 3) males without GnRH and females with GnRH (MOG/FWG), 4) males with GnRH and females without GnRH (MWG/FOG), and the rate of fertilization (%), viable embryos (%), and cause of death of fingerlings was analyzed. The results indicated an average of 99% of fertilized eggs in the study groups ($p > 0.05$), highlighting that MWG/FWG and MOG/FWG presented viable embryos higher than 60%; while the viability of MOG/FOG and MWG/FOG was less than 60%. The non-viable embryos were mainly associated with deformity. Once the eggs were hatched, the cause of death of fingerlings was determined, where MWG/FWG and MOG/FWG had a higher mortality by fungi and without apparent cause; while MOG/FOG and MWG/FOG, presented a higher mortality associated with deformity, being mainly the torsions in the spine the most outstanding, followed by defects in the resorption of the vital sack and other malformations at head and fin level. In conclusion, the use of GnRH in male and female breeding improves the quality of offspring by decreasing the number of unviable embryos and deformed juveniles.
Acknowledgments: We would like to thank Hendrix Genetics for providing animal resources. Post-doctorate FONDECYT/CONICYT Project N° 3170193.

Índice

29ª Reunión Anual
Sociedad Chilena de Reproducción y Desarrollo

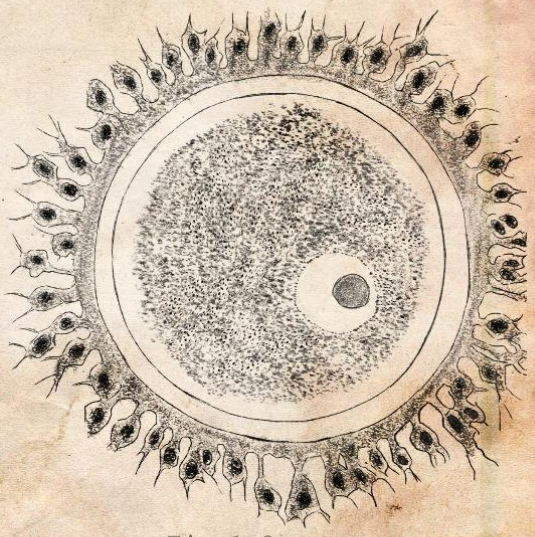

Fig.1:Ovum

5 al 8 de Septiembre, 2018

Hipocampus Resort & Spa
Concón-Chile

Fig.2:Sperm

Recepción de resúmenes
hasta el 29 de junio
secretaria.schrd@gmail.com

on, yr	1.0 ± 0.02	7.8 ± 0.1*	7.9 ± 0.1*
s, kg	—	—	10.4 ± 0.6
nass, kg	10.2 ± 0.3	11.2 ± 0.5	12.0 ± 0.8§
	50.0 ± 1.7	52.4 ± 3.5	61.2 ± 4.7
	18.3 ± 1.1	18.6 ± 1.6	19.6 ± 1.3
mass, g	68.3 ± 2.4	70.9 ± 5.0	80.8 ± 5.6