0265 - PLACENTAL APOPTOSIS ENHANCED BY HIF1 ALPHA STABILIZATION IS COUNTERACTED BY LEPTIN

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Abstract/Resumen: Leptin is a pleiotropic hormone produced by the placenta where it plays important regulatory functions. We have previously demonstrated that leptin promotes proliferation and survival of trophoblastic cells. Moreover, leptin prevents cellular stress under hypoxic condition in trophoblastic cells. In this work we aimed to study the mechanisms that mediate the effect of leptin in placental apoptosis induced by cobalt chloride ($CoCl_2$), a hypoxia mimicking agent that stabilizes HIF-1 alpha transcription factor expression. For this study we use Swan-71 cells, a first trimester trophoblastic human cell line, cultured under standard conditions, as well as human term placental explants. Swan-71 cells and placental explants were treated with $CoCl_2$ (50 or 100 µM). The expression of HIF-1 alpha, p53, Caspase-3 and cPARP was determined by Western blot or Immunofluorescence (IF). Apoptosis was determined by DNA ladder assay in placental explants. All procedures were approved by ethical review committee at the Alejandro Posadas National Hospital. We observed that HIF-1 alpha stabilization increased DNA fragmentation in placental explants (*p<0.05). Leptin treatment blocked this effect (#p<0.05 relativized to control treated with $CoCl_2$). On the other hand, treatment with $CoCl_2$ increases clivated PARP and Caspase-3 levels in a dose-dependent manner indicating that apoptosis was induced (**p<0.01). Moreover, p53 protein expression, a key regulator of apoptosis pathway, was enhanced by hypoxic condition (**p<0.01). We also observed that HIF-1 alpha stabilization increased nuclear p53 localization. All these results suggest that HIF-1alpha stabilization enhances placental apoptosis and leptin is capable to protect these cells under hypoxia conditions.

0374 - PREVALENCE OF ESCHERICHIA COLI AND ANALYSIS OF VIRULENCE FACTORS IN ENDOCERVICAL CULTURES FROM PREGNANT WOMEN.

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Abstract/Resumen: Presence of E. coli in the endocervical microbiome has been associated to pregnancy complications. We have previously reported that Shiga toxin (Stx) producing E. coli (STEC) infections during pregnancy may cause maternal or fetal damage mediated by Stx2 in rats in early stage of gestation. Moreover, Stx2 inhibits migration, invasion and cell viability in extravillous trophoblast human cells of first trimester. Therefore, we propose to study the presence of STEC in female genital tract in the pregnant women since might be risk factor during gestation. Our objective was to identify different virulence factors of STEC cultures of endocervix of pregnant women. Endocervical swabs from 103 asymptomatic pregnant women with gestational age of 14 to 30 weeks from the National Hospital Posadas were enrolled. Samples were enriched in Tryptic Soy Broth and sub-cultivated on sorbitol-MacConkey (SMAC) agar in order to detect no sorbitol fermenting colonies, characteristic of STEC. Genomic DNA was purified from colonies and the presence of the uidA gene, exclusive for E. coli was analyzed by polymerase chain reaction (PCR). Positive colonies for uidA were checked for rfbO157, lpfAO113, hcp, eae, stx1, stx2-2a genes. STEC strains positive for stx2 genes were also cultured in the presence of mitomycin C (2 µg/ml) to evaluate expression of Stx2 by viability assays on Vero cells. The PCR results showed that 16/103 samples were positive for SMAC agar and 15/103 were positive for the uidA gene. Furthermore, 6/15 E. coli expressed lpfAO113 and hcp, and 9/15 E. coli expressed stx2 being only one sample positive for stx2a variant. All of them were negative for rfbO157, eae and stx1 genes. One STEC strain positive for stx2 gene showed cytotoxic effects even in absence of mitomycin C. These results suggest that STEC strains could colonize the endocervix of pregnant women

0405 - VASOACTIVE INTESTINAL PEPTIDE (VIP) AS AN OVARIAN PROTECTOR: PREVENTION AGAINST PREMATURE OVARIAN FAILURE DURING CHEMOTHERAPY

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Abstract/Resumen: The ovary, in addition to its endocrine and intraovarian control, is regulated by direct neural inputs of peptidergic nature. Vasoactive intestinal peptide (VIP) was originally isolated from the small intestine and lung tissues and plays an important role in ovarian function. Previous studies have found VIP immunoreactivity in ovarian follicles. The objective of this study was to determine the effect of VIP on ovarian function in a doxorubicin (DX) induced-premature ovarian failure (POF) murine model. To induce POF, DX 10 mg/kg, i.p. was applied in F1 mice (C57XBalbC 8 weeks old) on day 1. Control and DX mice underwent sham surgery and received an intrabursal injection of saline solution on both ovaries, while DX + VIP groups received either 1 µl or 10 µl VIP 1 µM 1 h prior to DX administration. Sacrifices were made at day 15. The ovaries were isolated for histological analysis and protein extraction for Western Blot assays. For all data analysis ANOVA followed by Tukey test were performed. An ovarian morphological analysis showed that DX decreased the % of primary (PriF), preantral (PF) and early antral follicles (EAF), and increased the % of atretic follicles (AtrF) (p<0.05). VIP (1 µl) increased the % of EAF and decreased the % AtrF. However, the highest dose of VIP (10 µl) increased the % of PriF, PF and EAF, and decreased the % of AtrF compared to DX (p<0.05). These results were corroborated by IHC for Anti-Müllerian Hormone (AMH), where it was found that DX reduced the % of follicles expressing AMH, while VIP (both doses) increased it (p<0.001). DX increased the apoptotic index (cleaved caspase-3-positive follicles/total follicles) in follicles, compared to control (P<0.01). VIP (both doses) protected follicles from this increment. In conclusion, VIP might be a promising strategy to protect female fertility in cancer patients. Further studies on VIP effects on female reproduction in chemotherapy-induced POF and on the safety of use of this peptide are required.

0443 - CANNABINOID RECEPTOR 1 (CB1) IS INVOLVED IN PRETERM BIRTH INDUCED BY LPS

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Abstract/Resumen: Endocannabinoid system (ECs) is one of several signaling pathways implicated in maternal-fetal interface, and endocannabinoids are implicated in different aspects of physiopathology of reproduction. Preterm birth (PTB) is the

leading cause of mortality and morbidity in neonates. It is well known that premature deliveries are mainly associated with infectious process. In mice, it has been shown that one of the major causes of PTB is premature decidual senescence, which becomes more aggravated by an inflammatory stimulus. Our group developed a murine model of preterm labor, consisting of two injections of bacterial lipopolysaccharide (LPS) that produces an 85% of PTB in BALB/c mice. The aim of the present work was to evaluate if the ECs participates in LPS-induced preterm labor. For this purpose, we administrated two doses of bacterial lipopolysaccharide (LPS, 10 µg/g of weight and 3 h later 20 µg/g of weigh respectively) on day 15 of pregnancy to CD1-wild type mice (CB1-WT) and CD1-knock out mice for the cannabinoid receptor type one (CB1-KO). We found that CB1-KO mice show lower PTB percentage than CB1-WT mice (60 CB1-KO vs. 81 % CB1-WT). We studied different inflammatory mediators in decidua 5h after the second dose of LPS and observed that protein levels of TLR-4 were decreased in LPS treated mice ($p<0.05$) while CD14 and COX-2 protein levels were augmented ($p<0.05$). The same response pattern was observed both in CB1-WT and CB1-KO mice. It has been reported that disruption of autophagy balance (either increase or decrease) can lead to PTB. We evaluated decidual protein expression of LC3b II, a marker of autophagy, and observed that CB1-KO mice presented lower decidual protein levels of LC3b II when compared to CB1-WT ($p<0.05$). Considering the cross-talk between autophagy and senescence, we evaluated the protein expression of H2AX, an indicator of DNA damage, and did not observe differences between genotypes. In summary, our results indicate that cannabinoid receptor type one is involved in the triggering of LPS-induced preterm birth.

0776 - PRO-INFLAMMATORY AGENTS NITRIC OXIDE AND TNF ALPHA ARREST GC-1 SPERMATOGONIA CELL CYCLE THROUGH DIFFERENT MECHANISM

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Abstract/Resumen: Nitric oxide (NO) and tumor necrosis factor alpha (TNF alpha) are pro-inflammatory agents able to interfere with cell cycle. Experimental autoimmune orchitis (EAO) is a model of chronic inflammation associated to infertility. In EAO high levels of NO and TNF alpha are produced by testicular macrophages and pre-meiotic germ cells (spermatogonia and pre-leptotene spermatocytes) proliferation is reduced. We propose that NO and TNF alpha arrest spermatogonial cell cycle in EAO. To evaluate this hypothesis, we explored the effect of DETA-NO (a NO donor) and TNF alpha on cell cycle and death on GC-1 spermatogonia cell line by flow cytometry. Both TNF alpha (50 ng/ml) and DETA-NO (2.0 mM), significantly increased the percentage of GC-1 cells in the S-phase and significantly reduced the percentage in the G1-phase of the cell cycle (propidium iodide incorporation, IP) also inducing cell apoptosis (Annexin V-FITC-IP assay) after 24 and 18 h of incubation respectively. Pre-incubation of GC-1 cells with a general antioxidant, N-acetyl-L-cysteine (NAC, 2.5 and 5.0 mM) significantly reduced DETA-NO effect on cell cycle arrest and apoptosis while NAC did not modify TNF alpha action. DETA-NO induced GC-1 cell cycle arrest and apoptosis was reverted after DETA-NO withdrawal unlike TNF alpha.

1085 - THE RESPONSE TO ENVIRONMENTAL THERMAL STRESS IS NOT SEXUALLY DIMORPHIC AND DEPENDS ON ANDROGENS IN THE INDUCTION OF SEX REVERSAL OF MEDAKA FISH

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Abstract/Resumen: In many fish species, environmental stressors (ES), like high temperatures (HT), induce sex reversal of genotypic females (GF) to phenotypic male. In a previous study, we elucidated that all begins in the brain through an early increase of crhb, with the concomitant increase of whole body cortisol level. The synthesis of androgen as a by-product of cortisol inactivation has been proposed in a fish with environmental sex determination, the pejerrey. Nevertheless, the participation of androgen in the sex reversal induced by an ES during the gonadal sex determination period (GSDP) has not been corroborated. First, we performed a microarray after incubate embryos to HT, and control temperature (CT), from fertilization to GSDP. Our results showed a whole stress effects, inducing the differential expression of 2,517 of 11,600 genes. Many of them related to glucocorticoid, *e.i.* crhb, gr2, and thyroid axis, *e.i.* tsh, tg, and iyd; both axes has been related with sex reversal. Moreover, the pathway of sex steroid was up-regulated in HT treated embryos, especially to androgen synthesis, *e.i.* hsd3b, cyo11b, hsd11b2 and hsd11b3. On the other hand, the estrogen pathway was down-regulated, e.i. cyp19a1a. Moreover, it is important to highlight those DEGs analysis between sexes at the same treatment did not display differences, assuming of both genotypes sexes response similarly to an ES. Finally, the participation of androgens in the sex reversal was analyzed with Flutamide (Flu), an androgen receptor antagonist. XX larvae incubated at CT showed only ovarian development, but XX individuals incubated at HT until hatching presented an increased sex reversal towards male; however, in the case of Flu treatment of embryos incubated at HT, the sex reversal percentage decreases in a dose-dependent manner. Therefore, our results are consistent with an androgen synthesis response to an environmental stress, with the concomitant testis development bias.

AACyTAL II

Chairs: Marco Brocca | Gabriel Pinto | Marina Snitcofsky

0034 - BOTRYOMYCOSIS IN NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ MICE: A FOCAL OUTBREAK IN AN EXPERIMENTAL COLONY

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Abstract/Resumen: NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ mice were purchased from Jackson Laboratory and maintained under conventional closed barriers in individually ventilated cages (Tecniplast®) with controlled temperature (20-22°) and relative humidity (50–70%) in a 12:12 h light: dark cycle. The animals were fed with autoclaved standard diet and carbon activated filtered water ad libitum. Cages were filled with sterile wooden chips and corn. The experimental protocols were approved by the IACUC. Mice (n=9; 6-12 months-old) included in patient-derived xenografts (PDX) studies exhibited solitary or multiple fibrose nodules of 0.50 cm, located near to the mouth and nose. The cases appeared randomly in cages for over five months. After clinical examination, differential diagnoses included bacterial or fungal infections, foreign body granuloma, sterile pyogranuloma