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expression and activity as germ cell differentiation proceeds.

Keywords: spermatogenic cells, sphingolipids, very-long-chain PUFA

(1600) **NONYLPHENOL INDUCES CYTOSKELETAL CHANGES AND RELEASE OF PROINFLAMMATORY MEDIATORS IN RAT SERTOLI CELLS *IN VITRO***

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Nonylphenol (NP), an alkylphenol present in plasticizers, is an endocrine disrupting chemical that is potentially dangerous for male reproduction in mammals including man. Because Sertoli cells (SC) provide structural and metabolic support to germ cells, in this study the hypothesis that exposures of SC to NP affect their metabolic functions and the production of bioactive molecules was evaluated. Primary cultures of SC were preincubated with [^{3}H]arachidonic acid (AA) to label their lipids and then were treated with NP. The NP exposures resulted in increased concentrations of free AA in cells and medium, indicating that such AA was released from [^{3}H]-labeled lipids ($p<0.01$). This lipid was mostly phosphatidylinositol, acted upon after activation of a protein kinase A (PKA)/cytoplasmic phospholipase A2 (cPLA2). In NP-exposed SC, an increase of diacylglycerols (DAG) also took place in both, cells and medium ($p<0.01$). Part of such DAG may have served as second messengers, since NP-increased DAG were associated with an augmented production of PGE2 and expression (mRNA) of COX2. Since the network of vimentin intermediate filaments is important for intracellular lipid transport, the effects of NP on the structure of this network in relation to the formation of cytoplasmic lipid droplets (LD) was studied. In NP-treated SC, the vimentin network was redistributed and the LD size was increased. The NP-dependent cytoskeletal redistribution was prevented by preincubation with H89, a PKA inhibitor. The formation of large LD was prevented by preincubation with either H89 or MEP, a PLA2 inhibitor, suggesting the participation of PKA and cPLA2 in LD biogenesis. We conclude that NP is involved in activating the proinflammatory pathway in SC, by providing the AA that is necessary for prostaglandin synthesis via PKA/PLA2 on the one hand, and by generating the DAG that is required as cofactor of the PKC-mediated activation of the NF- κβ/Cox-2 inflammatory pathway on the other.

Keywords: endocrine disruptors; (in)fertility; proinflammatory mediators; COX2

(389) **PARTICIPATION OF SIRT1 IN THE REGULATION OF SERTOLI CELL PROLIFERATION**

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Abstract: Sertoli cells (SC) provide the structural and nutritional support for germ cell development. Considering that each SC is able to support a limited number of germ cells, the final number of SC reached during the proliferative periods determines sperm production capacity in adulthood. In the rat, SC proliferate during fetal and neonatal periods and it is well known that FSH is the major SC mitogen; however, little is known about the mechanisms involved in the detention of SC proliferation, essential for the formation of blood-testis barrier and SC differentiation. Sirtuins (SIRT1-7) belong to a cellular energy sensor family of NAD^+-dependent enzymes with deacetylase activity. SIRT1, the most studied member, plays an important role in several processes ranging from cell cycle regulation to energy homeostasis. The aim of this work was to investigate whether SIRT1 activation participates in the cessation of SC proliferation. Mitotically active SC obtained from 8-day old rats were maintained under basal (B) conditions or stimulated with FSH 100 ng/ml in the absence or presence of Resveratrol (RSV, 50μM) a polyphenol that increases SIRT1 activity. BrdU incorporation and the expression of cyclins D and p21 and p27 (cell cycle inhibitors) by RT-qPCR were evaluated. Results are expressed as mean±SD (n=3, different letters indicate statistically significant differences, $P<0.05$). RSV decreased BrdU incorporation under basal and FSH-stimulated cultures (B: 10.9 ± 1.9^a, RSV: 3.1 ± 1.3^b, FSH: 21.4 ± 3.2^c, FSH+RSV: 2.1 ± 2.3^b; %BrdU-positive cells) and inhibited the FSH effect on cyclin D1 and cyclin D2 expression. In addition, RSV increased p21 and p27 mRNA levels (p21: RSV: 3.3±1.1*; p27: RSV: 1.9±0.3*; fold stimulation vs B, *$P<0.05$). Altogether, these results suggest that SIRT1 activation may be involved in the cessation of SC proliferation through the regulation of cyclins and cell cycle inhibitors expression.

Keywords: Sertoli, proliferation, Sirt1, resveratrol

(1079) **PEDF (PIGMENT EPITHELIUM DERIVED FACTOR) EXPRESION IN MEPC5 CELLS (MOUSE) AND IN MALE REPRODUCTIVE TRACT (WISTAR RATS) UNDER ANDROGEN REGULATION**

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IHEM - Conicet - UNCuyo

Abstract: Pigment epithelium-derived factor (PEDF) expression has been described in many organs as showing neurotrophic, anti-angiogenic, anti-apoptotic, anti-inflammatory, anti-oxidant and pro-cell survival properties. However, references to its activity in the male reproductive system are scarce, except in prostate cancer and the regulation of sperm conjugation in rat epididymis. We aimed to characterize the expression of PEDF in MEPC5 cells (mouse epididymal proximal caput cells) and in the male reproductive tract of Wistar rats and explore their hormonal regulation. We found that PEDF is expressed in MEPC5 by Immunofluorescence and over the epididymis, prostate and seminal vesicles by immunohistochemistry, but notably not in the testes. These results agree with those obtain by semi cuantitative RT-PCR. Androgen dependence of PEDF expression was evaluated by flutamide administration during 15 days to Wistar Rats. PEDF expression diminished along the male reproductive tract. This decreased expression was reversed after 30 days without flutamide administration. The epididymis is an essential organ in sperm maturation-storage. The role of PEDF in this physiological process has not been fully elucidated. But considering that in other systems PEDF has anti-apoptotic, anti-oxidants and pro-cell survival properties, its expression along the epididymis may be related to the protection of spermatozoa while they are stored.

Keywords: PEDF, male reproductive tract, MEPC5 cells, androgens.

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(162) **POSSIBLE ROL OF TESTICULAR TRANSFERRIN IN THE HOMEOSTASIS OF SEMINAL IRON**

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Testicular Transferrin (TTf) is a glycoprotein secreted by Sértoli cells and it is involved in the transport of iron to developing germ cells. Iron homeostasis is defined by mechanisms in which the intracellular concentration of this metal is maintained at adequate levels for cellular requirement but nothing enough to cause toxic effects. The study of DNA and sperm membrane (SM) plays a fundamental role in seminal evaluation. The aim of the present work is to investigate whether there is relationship between TTf levels and SM integrity and sperm DNA. Twenty semen samples were studied: 5 fertile controls (according to WHO 2010) and 15 from patients with different andrological pathologies. The variables studied were: Concentration of TTf versus % of spermatozoa with altered membrane and Concentration of TTf versus DNA integrity, among which the Pearson Correlation Coefficient was applied. The integrity of SM