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**CON LA PARTICIPACIÓN DE
SOCIEDAD ARGENTINA DE VIROLOGÍA (SAV)
ASOCIACIÓN ARGENTINA DE NANOMEDICINAS (NANOMED-ar)**

**14-17 de noviembre de 2018
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EDITORES RESPONSABLES

Claudia Pérez Leirós
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RESPONSIBLE EDITORS

**Claudia Pérez Leirós
Pablo Baldi
Alberto Crottogini**

havior of spermatogonia (SP) population in infertile patients displaying testicular immune cell infiltrates. Biopsies obtained from infertile patients (Hospital. Clínicas, CABA) with obstructive and non-obstructive azoospermia were classified according to morphological criteria in: normal spermatogenesis (N, n=7), hypospermatogenesis (Hypo, n=5) and severe hypospermatogenesis (SHypo, n=5). The number of immune cells was quantified by immunohistochemistry employing the leukocyte common antigen marker (CD45) and the number of SP (A dark, A pale and B) by morphological criteria in the same slides. The number of CD45 cells increased significantly in Hypo and SHypo biopsies vs N (Mean±SEM N:22.150±2.510, Hypo:69.090±9.398, SHypo:95.180±12.54, p<0.001). The total number of SP decreased significantly in SHypo vs N (Mean±SEM N:27.820±0.972; Hypo:23.800±1.420; SHypo:9.339±0.699, p<0.001). The number of SP A dark decrease significantly in Hypo and also in SHypo compare to N (Mean±SEM N:4.716±0.2759; Hypo:2.994±0.2599; SHypo:1.016±0.1732, p<0.001); the number of SP A pale and B SP decreased only in SHypo (Mean±SEM, SP Apale, N:17.220±0.811; Hypo:15.950±1.088; SHypo:5.746±0.457; SPB, N:1.630±0.181; Hypo:1.055±1.156; SHypo:0.164±0.051). The hormonal profile showed that serum LH, testosterone did not change, while FSH was increased in SHypo vs N (Mean±SEM N:6.614±1.889; Hypo:19.200±6.003; SHypo:25.200±7.185, p<0.05). We demonstrated that inflammation is associated to spermatogenesis impairment and alter SP kinetic behavior. SP A dark, a quiescent population, consider as "the true stem cells" of the testis is reduced in both Hypo and SHypo, suggesting that is a population very sensitive to the inflammatory microenvironment.

- 624. (107) ASSESSMENT OF THE ROLE OF L-GLUTAMINE IN THE REGULATION OF SERTOLI CELL PROLIFERATION**
 María Noel Galarido, Agustina Gorga, Gustavo Rindone, Eliana Pellizzari, María del Carmen Camberos, Selva Cigorraga, María Fernanda Riera, Silvina Meroni
Centro de Investigaciones Endocrinológicas "Dr. Cesar Bergadá" (CEDIE)

The final number of Sertoli cells (SC) reached during the proliferative periods determines sperm production capacity in adulthood. On the other hand, it is well known that nutrient availability plays a pivotal role in the decision of a cell to commit to cell proliferation. Crucial to the decision process is the mammalian target of rapamycin (mTOR), whose activity is controlled by, besides hormones such as FSH, the presence of amino acids (aa). L-glutamine (gln), the most abundant aa in the blood, plays an important role in proliferation in many cell types. However, the role of gln in the regulation of SC proliferation has not been studied yet. The aim of this work was to investigate whether SC depends on the presence of gln to proliferate. SC obtained from 8-day old rats were maintained in the absence or presence of gln (2.5 mM) and stimulated with FSH 100 ng/ml. BrdU incorporation, cyclin D2 (CCND2) expression by RT-qPCR and phosphorylated mTOR (P-mTOR) levels by western blot were evaluated. Results are expressed as mean±SD of three independent experiments (different letters indicate statistically significant differences, p<0.05). It was observed that gln increased BrdU incorporation (0 mM gln: 1.4±0.3^a; 2.5 mM gln: 4.1±0.6^b; 0 mM gln + FSH: 4.0±0.6^b; 2.5 mM gln + FSH: 13.0±2.6^c % BrdU-positive cells) and also enhanced FSH stimulus on CCND2 expression. In addition, gln increased P-mTOR levels (2.5 mM gln: 2.7±0.3^b; 0 mM gln + FSH: 3.6±0.4^c; 2.5 mM gln + FSH: 4.7±0.5^d fold variation P-mTOR/T-mTOR vs. 0 mM gln). These results suggest that gln might be necessary for the regulation of Sertoli proliferation possibly through the modulation of mTOR pathway. PICT2014-0945.

- 625. (222) REGULATORY ACTIONS OF MELATONIN IN RODENT SERTOLI CELLS**
 Soledad Paola Rossi, María Eugenia Matzkin, Ricardo Calandra, Artur Mayerhofer, Mónica Frungieri
IBYME-CONICET, Facultad de Medicina, Universidad de Buenos Aires. BioMedizinisches Centrum (BMC), Ludwig-Maximilians-University (LMU). Ciclo Básico Común, Universidad de Buenos Aires.

Melatonin acting through the hypothalamus and pituitary regulates testicular function. In addition, direct actions of melatonin at testicular levels have also been detected. Recently, we described a protective role of melatonin against free radical damage in testicular mast cells. The aim of this study was to investigate the participation of melatonin in the regulation of the oxidative state and lactate dehydrogenase (*Ldh*) expression in Sertoli cells. For this purpose, we used Sertoli cells isolated from 21 days old Syrian hamsters and the murine TM4 Sertoli cell line, both expressing the melatonergic receptor subtype 1.

After a 2 h incubation in the presence of the oxidizing agent tert-butyl hydroperoxide (TBHP; 500μM), reactive oxygen species (ROS) production from TM4 cells was significantly increased. This action was prevented by 1μM melatonin (fluorometric assay, arbitrary units, a.u.: control: 6761 ± 501^a; TBHP: 13084 ± 686^b; melatonin + TBHP: 9085 ± 326^c; X ± SEM; P<0.05).

Melatonin (1μM) also inhibited H₂O₂ generation in TM4 Sertoli cells (fluorometric assay, a.u.: control: 4032 ± 130.13^a; melatonin: 2965 ± 39.61^b; X ± SEM; P<0.05).

Moreover, after a 5 h incubation, melatonin (1μM) significantly induced protein expression of the antioxidant enzymes catalase and peroxiredoxin 1 in TM4 Sertoli cells (determined by Western blot; P<0.05).

In immature hamster Sertoli cells, while melatonin seems to decrease lipid peroxidation (TBARS assay), it significantly increases catalase and peroxiredoxin 1 protein expression (P<0.05).

One of the most important Sertoli cell functions is to provide energy substrates (i.e. lactate) to germ cells. *Ldh* a gene expression was significantly increased in the presence of melatonin in both TM4 Sertoli cells and immature hamster Sertoli cells (quantified by qPCR; P<0.05).

Our data, although preliminary, reveal that melatonin exerts an antioxidant effect in Sertoli cells. In addition, this indolamine positively influences *Ldh* expression thus promoting germ cell development.

- 626. (650) OLIVE OIL IMPROVES THE ALTERATIONS OF THE TESTICULAR CHOLESTEROL METABOLISM REGULATED BY SREBP-2 IN HYPERCHOLESTEROLEMIC RABBITS**
 Abi Funes, Regina Colombo, Layla Simón, Leandro Cortese, Tania Saez, María Monclus, María Cabrilla, Miguel Fornes
IHEM-CONICET

The relationship between hypercholesterolemia and reduced male fertility has been reported previously. Hypercholesterolemic rabbits (HCR) were associated with deleterious changes in semen and sperm cells: semen volume decreased and the sperm membrane became overloaded with cholesterol. Olive Oil (OO) supplementation (7% v/p) improved semen parameters affected by high fat diet. The increase in membrane cholesterol may be due to changes in the intracellular metabolism of this lipid. New Zealand White rabbits, 4 rabbits from each group, were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), plus 14% bovine grease (HCR) or 7% bovine grease plus OO (7%) (½HCR + ½OO). Within molecular regulation, SREBP (Sterol-Regulatory-Binding-Protein) is an essential protein for cholesterol homeostasis and membrane biogenesis. At 3 months of diet, SREBP-2 mRNA expression showed no significant changes between NCR and HCR assessed by RT-PCR. In contrast, protein expression, detected by western blot showed a significant increase in HCR testis. The target molecules of SREBP2: HMGCoAR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) R-LDL (low-density lipoprotein receptor) and ABCA-1 (ATP-binding cassette, sub-family A member 1) followed the same pattern. Interestingly, ½HCR + ½OO showed a recovery in the expression of the mentioned proteins. In addition, the SREBP2 protein was sub-cellularly detected in the testis by indirect immunofluorescence. Taken together, the changes in expression and location of the SREBP and downstream proteins in testis of HCR animals could be related to high fat diet and seminal consequences. Instead, OO was able to improve the altered parameters in dietary acquired hypercholesterolemic rabbits.

- 627. (474) EFFECT OF BIOACTIVE NATURAL COMPOUNDS**