

number negatively correlates with the number of immune cells in testicular biopsies of azoospermic patients. Proinflammatory agents nitric oxide (NO) and TNF α produced by the immune cells that infiltrate the testis might impair spermatogenesis. Our objective was to evaluate the effect of NO and TNF α on Spg and preleptotene spermatocyte (PLs) proliferation and spermatogenesis progression in adult *Wistar* rats. DETA-NOate (DETA-NO), a NO donor, or TNF α were injected in one testis, saline was injected in the contralateral testis. On day 5, a group of animals received BrdU injection (ip) and were euthanized 2h later in order to evaluate proliferation, by immunofluorescence. Another group was sacrificed on day 60 to evaluate the effect on spermatogenesis. DETA-NO (2 and 10mM) significantly reduced the number of BrdU+Spg/seminiferous tubule (ST) ($p < 0.05$, Student paired t test, $n=3$) and the number of BrdU+PLs/ST ($p < 0.01$; Student paired t test, $n=3$) vs saline. These events slowed seminiferous epithelial cycle, demonstrated by the significant reduction in the number of ST at VII-VIII stage ($p < 0.01$; Student t test, $n=3$). Variations in STs' area reflect the magnitude of spermatogenesis damage; after 60d DETA-NO (2 and 10mM) significantly increased the frequency of STs with small and reduced area respectively vs. saline ($p < 0.05$; Student t test, $n=3$). TNF α (0.1 and 1 μ g) exposure affects neither Spg nor PLs proliferation or spermatogenesis. We demonstrated that NO arrests the cell cycle of premeiotic GCs, limiting Spg mitotic amplification division and the entrance of PLs in meiosis. These events might generate time gaps in the spermatogenic wave lastly affecting sperm production.

525. (086) COMPARATIVE ANALYSIS OF SPERMATOGENESIS AND HORMONAL PROFILE OF INFERTILE PATIENTS WITH IDIOPATHIC ORCHITIS VERSUS RATS WITH AUTO-IMMUNE ORCHITIS

María Sofía Amarilla, Patricia Verónica Jacobo, Leilane Glienke, Cristian Marcelo Sobarzo, María Belén Maio, María Jimena Ferraris, Livia Lustig, María Susana Theas
Instituto de Investigaciones Biomédicas (INBIOMED), UBA-CONICET. Facultad de Medicina. Universidad de Buenos Aires, Argentina.

Experimental autoimmune orchitis (EAO) is a well-established rodent model of organ specific autoimmunity associated to infertility. Testis immunopathology is similar in rats and humans undergoing a chronic testicular inflammation. A comparative analysis of other aspects of the disease like the quantification of spermatogonia (Spg) and Sertoli cells (SCs), by immunohistochemistry, as well as the hormonal serum profile of infertile patients with idiopathic orchitis vs rats with EAO was undertaken (RIA). We evaluated testicular biopsies from patients with idiopathic non-obstructive azoospermia, diagnosed with hypospermatogenesis (mild: $n=8$, severe: $n=10$) (HypE) and Sertoli cell only syndrome (SCOS, $n=9$). All groups displayed twice the number of immune cells (CD45 $^{+}$) vs patients with obstructive azoospermia and complete spermatogenesis (control group, C, $n=8$). The number of undifferentiated and differentiated SPg/seminiferous tubule (ST) decreases in mild and severe HypE while the number of SCs/STs increases in severe HypE and SCOS vs. control ($p < 0.01$). In EAO undifferentiated Spg (CD9 $^{+}$) increased in focal and decreased in severe EAO vs. normal (N) rats. Differentiated SPg (c-Kit $^{+}$)/ST decreases (mean \pm SEM, N:10.5 \pm 0.3, focal EAO:4.4 \pm 0.1, severe EAO:3.1 \pm 0.3, $p < 0.05$, $n=3$) and the SCs/STs number increases vs. N (mean \pm SEM, N:10.14 \pm 1.13, focal EAO:17.32 \pm 2.24, severe EAO:19.5 \pm 3.5, $p < 0.05$, $n=3$). FSH, was higher in severe HypE vs C and also in severe EAO vs N. Testosterone and LH were similar to C in severe HypoE and also in severe EAO vs N. Prolactin in mild and severe HypoE was similar to C and in focal and severe EAO was similar to N (mean \pm SEM ng/ml, N:13.1 \pm 2.0, focal EAO:10.2 \pm 1.8, severe EAO:14.9 \pm 2.8, $n=11$). We showed that particularly the late stages of EAO closely reflect Spg and SCs behavior, and hormonal profile observed patients with severe HypE. These results validate EAO as a valuable model for studying the impact of inflammatory processes on spermatogenesis.

526. (106) EVOO RESTORES THE STEROL REGULATORY ELEMENT-BINDING PROTEIN 2 CHOLESTEROL PATHWAY OVER-STIMULATED BY A HFD IN RABBIT TESTIS

Abi Karenina Funes ¹, Virginia Avena ¹, Regina Colombo R ¹, María Monclus^{1,2}, Inés Conte¹, Paola Boarelli ³, Estefanía Saez Lancellotti ^{1,2}, Miguel Fornés ^{1,2}.

¹ Laboratorio de Investigaciones Andrológicas de Mendoza (LIAM), IHEM, Universidad Nacional de Cuyo, CONICET. ² Centro de Investigaciones de la Universidad del Aconcagua (CIUDA), Universidad del Aconcagua. ³ Laboratorio de Enfermedades Metabólicas (LEM), Universidad Maza.

Male fertility depends on cholesterol (chol) homeostasis. Chol is essential for testosterone synthesis and spermatogenesis, and must be maintained in an optimal range for proper functioning of the testes. Rabbits on a high-fat diet (HFD) exhibit hypercholesterolemia associated with poor seminal quality, related to cholesterol overload in seminiferous tubule cells. Sterol regulatory element-binding protein (SREBP)-2 governs the cholesterol pathway in testis and it is sensitive to dietary lipids. We have previously seen that Extra Virgin Olive Oil (EVOO) supplementation improved semen parameters affected by high fat diet. The aim of this study was to explore the effects of EVOO supplementation to HFD on rabbit testes at the molecular level, analyzing the SREBP-2 pathway. Male New Zealand White rabbits were fed commercial rabbit pellet (normocholesterolemic rabbits: NCR), a high-fat diet (plus 14% bovine grease, hypercholesterolemic rabbits, HCR), or 7% bovine grease plus 7% EVOO (HCR + EVOO). Serum lipid levels, body weight and seminal parameters were measured, and mRNA and protein levels of the SREBP-2 pathway were assessed by PCR, Western blotting and immunofluorescence. At 12 months of diet, HCR rabbits show an increase in the expression of SREBP 2 and downstream molecules of the pathway: HMGCR (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) and LDLR (low-density lipoprotein receptor). Interestingly, the addition of EVOO showed a recovery in the expression of the mentioned proteins. In addition, preliminary studies of SREBP-2 regulatory molecule, INSIG1 (Insulin induced gene 1), and the molecule responsible for the esterification of cholesterol, SOAT2 (Sterol O-Acyltransferase 2), showed no significant changes between diets so far. The data showed that dietary supplementation with EVOO promoted testicular improvements by modifying the expression of cholesterol pathway regulated by SREBP2.

527. (112) THE ENDOMETRIAL EXPRESSION OF INTERLEUKIN-1 FAMILY: THEIR INVOLVEMENT IN DELAYED CONCEPTION OF DAIRY COWS

Sofía Cainelli, María Belén Peralta, Emmanuel Angeli, Antonela F. Stassi, Natalia R. Salvetti, Hugo H. Ortega, Florencia Rey, Melisa ML. Velázquez
Laboratorio de Biología Celular y Molecular Aplicada. Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Universidad Nacional del Litoral (UNL) / Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), (3080) Esperanza, Santa Fe, Argentina

The cytokines of the interleukin-1 family are closely involved in processes such as resolution of uterine inflammation and are locally produced by macrophages and endometrial cells under stimuli. However, little is known about the role of these cytokines in the absence of disease during postpartum period where conception and pregnancy occur in cattle. The aim of this study was to analyse the gene and protein expression levels of the members of IL-1 family: IL-1 α , IL-1 β , IL-1RI, IL-1RII and IL-1RA during postpartum period, and their possible association with delayed conception. Endometrial biopsies were obtained from multiparous Holstein cows ($n=16$) at 45 and 60 days in milk (DIM). The voluntary waiting period of cows was 70 days. All procedures were approved by the Ethics Committee (FCV-UNL). The gene expression of IL-1 α , IL-1 β , IL-1RI, IL-1RII and IL-1RA was analyzed by real-time PCR. The immunolocalization of the cytokines was assessed by indirect immunohistochemistry.

Kaplan–Meier test was used to evaluate the possible association between the gene and protein levels of each cytokine and delayed conception. Then, when the results from Kaplan–Meier showed significant association, we grouped the animals like 'fewer number of days to conception' (FDC) and 'greater number of days to concep-