(1.9±0.3** and 1.8±0.3** fold change vs. B) after 24 h of treatment. Overall, these results suggest that ActA stimulates SC proliferation increasing c-Myc activity and cyclins expression as well as activating signaling pathways classically linked to cell cycle progression. (PICT:2015-228; 2018-1291).

521. (075) **PREVALENCE AND ASSOCIATION OF *CHLAMYDIA TRACHOMATIS*, *UREAPLASMA* SPP. AND *MYCOPLASMA HOMINIS* UROGENITAL INFECTIONS IN PATIENTS WITH PRIMARY INFERTILITY**

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Currently, infertility affects 15-20% of couples of reproductive age worldwide, with women and men equally contributing to infertility cases. Among others, urogenital infections are known causes of infertility. In fact, infertility have been associated to *Chlamydia trachomatis* (Ct), *Ureaplasma* spp. (Uu) and *Mycoplasma hominis* (Mh) urogenital infections. However, evidence from large studies assessing their prevalence and putative associations in patients with infertility is still scarce. Herein, we aimed to evaluate the prevalence and associations of Ct, Uu and Mh infection in women and men seeking care for infertility. A cohort of 5464 patients with a diagnosis of couple's primary infertility and 404 control individuals were enrolled. Cervical-swab and semen samples were collected from female and male individuals, respectively, and infections assessed by PCR or culture. Overall, the prevalence of Ct, Uu and Mh urogenital infection was significantly higher in patients than in control individuals (5.3%, 22.8% and 7.4% versus 2.0%, 17.8% and 1.7%, respectively). Ct infection was more prevalent in male than in female patients (OR: 1.36, *p=0.034*), being males younger than 25 years at the highest risk (OR: 2.51, *p=0.002*). Conversely, Uu and Mh infections were more prevalent in female patients, since males were less likely at risk of Uu (OR: 0.52, *p<0.001*) and Mh (0.41, *p<0.001*) infection. In addition, Uu infection was more prevalent in patients younger than 25 years, either in women (OR: 2.27, *p=0.003)* or men (OR: 1.66, *p=0.034*). Finally, a significant association between Mh and Uu infections was found in either female (OR: 33.84, *p<0.0001*) or male (OR: 71.83, *p<0.0001*) patients. Our data revealed that Ct, Uu and Mh are prevalent uropathogens in patients with couple's primary infertility. Taken together, our results show the importance of including the screening of urogenital infections in the diagnostic work up of male infertility.

522. (077) **INTERFERON γ, IL-17, AND IL-1B IMPAIR SPERM MOTILITY AND VIABILITY AND INDUCE SPERM APOPTOSIS**

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Urogenital inflammation is a known cause of male infertility. Increased levels of inflammatory cytokines, leukocyte counts and oxidative stress are highly detrimental for sperm quality thus compromising male fertility. Although cytokines affect sperm by recruiting and activating leukocytes consequently inducing oxidative stress, scarce to absent data have been reported about the putative direct effects of inflammatory cytokines on spermatozoa. Herein, we analyzed whether IFNγ, IL-17, IL-1β, and IL-8 can directly impair human sperm motility and viability. Fractions of viable and motile spermatozoa from normospermic healthy donors were in vitro incubated with recombinant human IFNγ, IL-17, IL-1β or IL-8 and sperm motility, viability and apoptosis were analyzed. Sperm exposed to different concentrations of IFNγ, IL-17 and IL-1β, or a combination of them, for either 1 or 3 h showed significantly reduced motility and viability with respect to sperm incubated with vehicle. Moreover, the exposure to IFNγ, IL-17 and IL-1β resulted in significantly higher levels of early and/or late apoptotic and/or necrotic spermatozoa. Interestingly, no significant differences in sperm motility, viability and apoptosis were observed in sperm incubated with different concentrations of IL-8, for either 1 or 3 h, with respect to sperm incubated with vehicle. In conclusion, our results indicate that IFNγ, IL-17 and IL-1β directly impair sperm motility and decreases viability by inducing sperm apoptosis. Our results suggest that examining inflammatory cytokines in semen would be an additional helpful tool for the diagnostic workup of male infertility.

523. (083) **NEW ADVANCES IN THE USE OF MELATONIN AS A FERTOPROTECTIVE AGENT DURING CHEMOTHERAPY**

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Melatonin (MEL) is a neurohormone with a potent antioxidant activity. Premature ovarian failure (POF) is a pathology defined as the depletion of the ovarian reserve; one of its causes is chemotherapy. Currently treatments for POF have proven to be ineffective. The aim of the present study is to assess whether MEL can protect ovaries from chemotherapy-induced gonadotoxicity using a mice model of POF induced by cyclophosphamide (CTX). Previously, we have reported that MEL enhances the % of antral follicles, diminishes the % of atretic follicles and increases SOD1 expression in POF model. To induce POF, CTX was administered (75 mg/kg, i.p.) to F1 mice (C57XBalbC, 6-8 weeks old) on day 1. CTX+MEL group also received MEL (15 mg/kg, i.p.) on days 1, 6 and 11. Animals were sacrificed on day 15 and their ovaries processed for histological analysis. Data was analysed by ANOVA followed by Tukey's test.
Histopathological analysis of ovarian sections showed that CTX caused fibrotic foci and blood vessel hyalinization; MEL decreased these parameters. IHC for AMH (ovarian reserve marker) showed that CTX diminished the % of follicles expressing AMH compared to control ($p<0.05$), whereas MEL increased this parameter compared to CTX ($p<0.05$). IHC for DDX4 (oocyte marker) revealed that CTX diminished the number of primordial follicles compared to control ($p<0.05$), whereas MEL increased it ($p<0.05$). IHC for CD31 (endothelium marker) revealed that CTX reduced the blood vessel density compared to control ($p<0.05$), while IHC for α-SMA (vascular stability marker) showed that CTX reduced mature vessel density ($p<0.05$). MEL increased these parameters compared to CTX ($p<0.05$).
In conclusion, these results, combined with our previous report, suggest that MEL could protect ovarian function from gonadotoxicity, preventing primordial follicle loss and restoring vascular function. MEL might represent a non-invasive treatment to preserve female fertility in patients undergoing chemotherapy.

524. (084) **NITRIC OXIDE (NO) BUT NOT TNF ALPHA INHIBITS GERM CELL CYCLE PROGRESSION AND IMPAIRS RAT SPERMATOGENESIS**

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Low spermatogenic efficiency in infertile men is not only due to post meiotic events, but also to decreased meiotic activity and spermatogonia (Spg) number. We demonstrated that Spg decreased

number negatively correlates with the number of immune cells in testicular biopsies of azoospermic patients. Proinflammatory agents nitric oxide (NO) and TNFα produced by the immune cells that infiltrate the testis might impair spermatogenesis. Our objective was to evaluate the effect of NO and TNFα on Spg and preleptotene spermatocyte (PLs) proliferation and spermatogenesis progression in adult *Wistar* rats. DETA-NOnoate (DETA-NO), a NO donor, or TNFα 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 immunofluorescense. 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 of small and reduced area respectively vs. saline ($p<0.05$; Student t test, n=3). TNFα (0.1 and 1μg) 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 AUTOIMMUNE ORCHITIS**

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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±SEM, N:10.5±0.3, focal EAO:4.4±0.1, severe EAO:3.1±0.3, $p<0.05$, n=3) and the SCs/STs number increases vs. N (mean±SEM, N:10.14±1.13, focal EAO:17.32±2.24, severe EAO:19.5±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±SEM ng/ml, N:13.1±2.0, focal EAO:10.2±1.8, severe EAO:14.9±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**

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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**

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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-1α, IL-1β, 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-1α, IL-1β, 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-