07. Endocrine, Reproductive and Urogenital Pharmacology

07.001 Spermatic evaluation in rats submitted to neonatal leptin treatment, a model of maternal malnutrition during lactation. Maia IC, Gontijo LS, Marques EB, Ribas JAS, Scaramello CBV, Marostica E UFF – Fisiologia e Farmacologia

Introduction: Neonatal leptin treatment, a model of maternal malnutrition during lactation, programs both hyperleptinaemia and hyperinsulinaemia in adulthood, which leads to leptin resistance by reducing the expression of the hypothalamic leptin receptor (Toste *et al*. Br J Nutrition, v.95, p.830, 2006). In adults, leptin stimulates gonadotrophin-releasing hormone (GnRH) secretion (Yu *et al.,* Proc Natl Acad Sci, v.94, p.1023, 1997) and can exert indirect effects on the male reproductive tract. Thus, the aim of this study is evaluate sperm functionality in this experimental model in rat. **Methods:** The use of animals was according to Ethics Committee (CEPA/UFF 0389/13). Male Wistar pups were divided into two groups: Leptin (L group) and Control (C group). During lactation (first 10 days) the animals were treated with leptin (8µg/100g sc) (L group) or saline (C group). After weaning, functional assays were performed in rats at 1, 3, 5 and 12 months-old. After each experimental period, the animals were anesthetized, testes and epididymis fat from different experimental groups were removed and weighed and spermatic evaluation (progressive motility, vigor, membrane integrity and hypo-osmotic swelling test) was performed using sperm from epididymis cauda. The values are mean±SEM, Student "*t*" test, $P<0.05$. **Results:** Body weight was higher in L group from 1^{th} to 5^{th} (1^{th}: C=109.9±2.3, L=120.6±2.3; 3^{th}: C=334.9.1±5.7, L=361.3±4.1; 5^{th}: C=386.9±7.7, L=426.9±5.8 g), but not at the 12^{th} (C=540.7±14.6, L=527.0±10.6 g) months-old when compared to C group. Gonadosomatic index (medium testes weight/body weight) decreased in L group at the 3^{th} and 5^{th} (3^{th}: C=0.52±0.01, L=0.46±0.02; 5^{th}: C=0.46±0.01, L=0.41±0.01), but it was higher at the 12^{th} (C=0.36±0.01, L=0.42±0.01) months-old. Epididymis fat increased after the 5^{th} months-old (5^{th}: C=1.45±0.18, L=1.99±0.13; 12^{th}: C=2.54±0.11, L=3.58±0.20). On the other hand, leptin treatment has did not affect the majority of spermatic evaluation parameters (progressive motility, vigor and hypo-osmotic swelling); only the membrane integrity improved in L group in 12^{th} months-old compared to C group the same age (C=37.17±3.8, L=48.1±1.35 %). **DISCUSSION**: Hormonal and nutritional influences during the neonatal period can change physiological and metabolic parameters of offspring in adulthood. Since this experimental model of malnutrition during lactation increases leptin serum concentration, this hormone can change the control of body weight in these puppies. Our data suggest that the increase in serum leptin decreases testicular atrophy resulting from aging, increasing periepididymal adipose tissue as a compensatory mechanism to the intake of sex steroids in this tissue. Furthermore, the membrane integrity improved can be direct or indirect leptin interference on protein synthesis, altering epididymis proteins related to sperm membrane composition in long term. Other experiments should be done to confirm these hypotheses. In conclusion, our preliminaries results suggest that neonatal leptin treatment to male rats not impair sperm functionality in an important manner in this experimental model. **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF. **Keywords:** Leptin; metabolic programming; spermatic evaluation

07.002 Soluble guanylyl cyclase activation by BAY 58-2667 improves bladder function in a mouse model of interstitial cystitis. de Oliveira MG, Calmasini FB, Alexandre EC, De Nucci G, Mónica FZ, Antunes E FCM-Unicamp – Farmacologia

Introduction: Interstitial cystitis (IC) is a chronic inflammatory disorder characterized by suprapubic pain, discomfort, excessive urgency and urinary frequency, besides profound effects on patient's quality of life[1]. Cyclophosphamide (CYP) is commonly used as an experimental model for the investigation of IC because of its similarity with the human disease[2]. Studies demonstrate that extensive oxidative stress during inflammation compromise bladder function by impairing the nitric oxide (NO)-soluble guanylyl cyclase (sGC)-cGMP signaling. Activators of sGC interact directly with its prosthetic heme group, enhancing the enzyme responsiveness in pathological conditions[3]. This study aimed to evaluate the effects of the sGC activator BAY 58-2667 on voiding dysfunction, protein expressions of α_1 and β_1 sGC subunits and cGMP levels in the bladder tissues after (CYP) exposure. **Methods:** All animal procedures and experimental protocols were approved by the Institutional Committee for Ethics in Animal Research/University of Campinas (protocol number: 3508-1). Female C57BL/6 mice (20-25 g) were injected with CYP (300 mg/kg, i.p) to induce cystitis. Mice were pretreated or not with BAY 58-2667 (1 mg/kg, gavage), given 1 h prior to CYP injection. The micturition patterns and in vitro bladder contractions were evaluated at 24 h. Protein expressions of sGC α_1 and β_1 subunits and COX-2 were obtained by western blotting. Reactive-oxygen species (ROS) were measured by DHE technique. **Results:** In freely-moving mice, CYP injection produced reduced the micturition volume and increased the number of urine spots. Cystometric recordings in anaesthetized mice revealed that CYP injection significant increases the basal pressure, voiding frequency and non-voiding contractions (NVCs), along with decreases in bladder capacity, intercontraction interval and compliance. BAY 58-2667 significantly prevented the micturition alterations observed in both freely-moving mice and cystometry, and normalized the reduced in vitro carbachol-induced contractions in CYP group. Reduced protein expressions of α_1 and β_1 sGC subunits, and of cGMP levels were observed in CYP group, all of which were prevented by BAY 58-2667. CYP exposure significantly increased the ROS generation in both detrusor and urothelium, and this was normalized by BAY 58-2667. The increased MPO and COX-2 activities in the bladders of CYP group remained unchanged by BAY 58-2667. **Conclusion:** Activators of sGC may constitute a novel and promising therapeutic approach for management of interstitial cystitis. **References:** [1]Hanno PM, et al. J Urol 193: 1545-1553, 2015; [2]Golubeva A, et al. Physiol Rep. 2(3): e00260, 2014; [3]Hoffman LS, et al. Br J Pharmacol. 157(5): 781-95, 2009. **Financial Support:** FAPESP, CNPq

07.003 How important is Alpha-1 adrenoceptor in primate and rodent proximal urethra?
Alexandre EC, Oliveira MG, Campos R, Kiguti LR, Calmasini FB, Silva FH, Antunes E – FCM-Unicamp – Farmacologia

Introduction: The lower urinary tract (LUT) consists of the urinary bladder and urethra that functions in a coordinated activity during micturition cycle voiding and storage phases. Sympathetic neural system activation leads to noradrenaline release, resulting in bladder relaxation and urethral contraction. Urethral smooth muscle (USM) contributes to urinary continence by contracting during the urine storage phase, which is mediated by activation of post-junctional α-1 adrenoceptors (Michel et al., 2006). Males and females greatly differ regarding the functioning of the LUT under physiopathological conditions, which may in part reflect the anatomy and morphology of USM (Pradidarcheep et al., 2011). However, few studies have attempted to characterize the gender differences in urethra. Therefore, this study was designed to evaluate the α1-adrenoceptor-mediated contractile responses and receptor subtype, as well as the tyrosine hydroxylase expression in USM from males and females mice and nonhuman primates (marmoset). **Methods:** Mice (C57BL6/J) and marmosets (Callithrix Jacchus) were killed under anesthesia, and proximal urethra excised. USM rings were mounted in organ baths containing Krebs-Henseleit solution (95% O2 / 5% CO2). Concentration-response curves to phenylephrine (10 nM to 3 mM) and noradrenaline (10 nM to 300 µM), and contractile responses to electrical-field stimulation (EFS; 1-32 Hz, 80 V) were conducted. qRT-PCR for α_{1A}, α_{1B} and α_{1C} adrenoceptor subtypes and tyrosine hydroxylase as markers for sympathetic pathway were performed. A histomorphometric analysis was made to evaluate mouse USM area. **Results:** Phenylephrine produced concentration-dependent USM contractions in male mice and marmosets (Emax: 5.7 ± 0.19 and 5.4 ± 0.73 mN; pEC50: 5.4 ± 0.03 and 4.5 ± 0.11 mN, respectively). Noradrenaline also produced concentration-dependent USM contractions in male mice. However, in female mice and marmosets, discrete USM contractile responses were observed. Pre-incubation of the α_{1A} adrenoceptor selective antagonists tamsulosin (0.3 nM) and RS 100329 (1 nM) significantly reduced phenylephrine-induced USM contractions in male mice, whereas the α_{1B}- and α_{1D} selective antagonists L 765,314 and BMY 7378 failed to affect the contractions. The α_1 adrenoceptor mRNA quantification revealed a marked presence of α_{1A} subtype in USM of males, with little expressions of α_{1B} and α_{1D} in either group. Mouse male urethra also exhibited marked higher tyrosine hydroxylase mRNA expression and smooth muscle area than female urethras. **Conclusion:** Female proximal urethra exhibits a poor sympathetic control, whereas male urethras contain sympathetic innervation and a rich population of α_{1A} adrenoceptors acting as the main contractile receptor. This finding may help future studies concerning the importance of urethra to the micturition cycles in females and males, and pharmacological strategies to treat LUT disturbances in both genders. **References:** Michel et al., (2006) 147 2: S88–119. Pradidarcheep et al., (2011) 117–148. **Financial Support:** FAPESP 2014/02196-2. **Animal Research Ethical Committee:** Mice (CEUA#3510-1) and marmosets (CEUA#3811-1/Sisbio #16951-1)**.**

07.004 Sexual dysfunction of hypertensive female rat improved with chronic ipriflavone treatment in both youth and senescence. Martins TA, Mendes JC, Rodovalho GV, Grabe-Guimarães A, Mosqueira VCF, Leite R UFOP – Ciências Farmacêuticas

Introduction: Female sexual arousal disorder (FSAD) is a major subcategory of female sexual dysfunction (FSD). A decline in serum estrogen, which occurs in menopause, results in a significant decrease in the clitoral intracavernosal, vaginal, and urethral blood flow, thinning of vaginal mucosal epithelium and atrophy of vaginal wall smooth muscle. The influence of estrogen is closely related to the local action of nitric oxide (NO) in the genital tissues. The conventional hormone therapy has proven effective in the treatment of FSD, however, this therapy is associated with an increased risk of the incidence of ischemic cardiovascular events, as well as venous embolism. Therefore, there is great interest in the development of therapeutic alternatives that minimize the deleterious effects of hypoestrogenism on sexual function without causing side effects and avoiding contraindications. The phytoestrogen ipriflavone (7-isopropoxy-3-phenyl-chromen-4-one), is used in the prevention and treatment of osteoporosis, common in the menopause/climacteric. However, the potential role of ipriflavone in the treatment of FSAD is unknown. The aim of this study was to investigate the therapeutic potential of ipriflavone in the treatment of FSAD associated with hypertension and senescence. **Methods:** For that, we used young (4-5 months) and senescent (18-19 months) female spontaneously hypertensive rats (SHR) treated for 30 days with vehicle, 10 or 30 mg/Kg of ipriflavone conveyed in a self-emulsifying drug delivery systems (SEDDS), which increases the bioavailability of the phytoestrogen. Physiological parameters were evaluated during pelvic nerve stimulation. Differences were considered statistically significant when $p<0.05$ using ANOVA followed by Bonferroni post-test for a number of 10 experiments per group. **Results:** Intravaginal temperature significantly increased at the dose of 10 mg/Kg in the senescent SHR group. The vaginal lubrication was improved after treatment with ipriflavone 30 mg/Kg in the young and in the senescent rats. Intravaginal pressure was significantly higher in both young and senescent groups treated with either 10 or 30 mg/Kg of ipriflavone. Chronic treatment with ipriflavone with doses of 10 and 30 mg/kg improved the genital vasocongestive responses observed during the subsequent 30 minutes interval after subcutaneous injection of apomorphine (80µg/kg) in conscious young and senescent female SHR. Furthermore, using the same model to study sexual function in conscious rats, the improvement in the genital vasocongestive responses observed in ipriflavone treated rats was similar to that observed for the estradiol treated group. Histological evaluation of liver and kidney, along with the characterization of the lipid profile and some enzyme levels (kidney and liver enzymes) suggest the absence of hepatic and renal toxicity. **Conclusion:** Chronic treatment for 30 days with the phytoestrogen ipriflavone conveyed in SEDDS, showed to be safe and significantly ameliorated female sexual dysfunction of young and senescent hypertensive rats, suggesting a therapeutic potential for the treatment of the sexual dysfunction induced by hypoestrogenism and potetiated by hypertension observed in menopause/climacteric woman. **Financial support**: FAPEMIG, CNPq and CAPES. (CEUA-UFOP: 2013/15).

07.005 Pregnant rats treated with dexamethasone show altered lipid metabolism during lactation. Mesquita FPN[1], Teixeira CJ[1], Souza DN[1], Santos-Silva JC[2], Veronesi VB[1], Ferreira DS[1], Silva PMR[1], Murata G[2], Anhê GF[1], Bordin S[2] [1]FCM-Unicamp – Farmacologia, [2]USP – Biofísica e Fisiologia

Introduction: Breastfeeding is a singular period of maternal life during which high quantities of maternal substrates are transported to the offspring through the milk. Although the mammary gland has prominent capacity to perform *de novo* fatty acid synthesis, much of the fatty acid composing milk triacyl-glycerol (TAG) is derived from circulating NEFA and from lipase lipoprotein (LPL)-mediated hydrolysis of TAG pools with chylomicrons and VLDL. Coordinated changes in metabolic active tissues such as white adipose tissue (WAT) and liver also take place to assure proper flux of substrates to the mammary gland. During breastfeeding, these metabolic routes are under control of many endocrine signals, such as glucocorticoids and prolactin. With respect to glucocorticoids, high doses of synthetic glucocorticoids (i.e.: betamethasone and dexamethasone) are often used in preterm mothers to avoid respiratory distress syndrome in the infant. However, it is still not known whether this therapeutic intervention impacts the lipid metabolism of the lactating mother. **Methods:** Circulating and hepatic maternal TAG levels were assessed in lactating rats treated with vehicle or dexamethasone (DEX) during pregnancy. Samples were collected 3, 7, 14 and 21 days after delivery. TAG levels were also assessed in mammary glands. Maternal milk was collected for TAG determination 3 days after delivery. Lactating mothers (at the third day after delivery) were also subjected to VLDL production assay (challenge with tyloxapol) and lipid absorption assay. Mammary glands were also processed for RNA extraction and PCR analysis of LPL. **Results:** DEX treatment during pregnancy increased circulating TAG but not cholesterol levels during lactation (throughout the entire lactation period). This increase was not accompanied by changes in hepatic TAG content or by expression of genes related to TAG synthesis and VLDL assembly and secretion. Our data also reveal that lactating rats treated with DEX show increased TAG levels after challenge with tyloxapol, suggesting reduced TAG removal by mammary gland. Accordingly, lactating rats treated with DEX displayed increased circulating TAG levels after oral load with olive oil as compared to lactating rats treated with vehicle. Treatment with DEX also yielded increased TAG levels in mammary and reduced TAG levels milk. The positive effect of lactation on CIDEA (cell death inducing DFFA-like effector A) mRNA expression was suppressed by DEX treatment. **Conclusion:** Altogether our data suggest that treatment with DEX during pregnancy results in reduced ability of mammary gland incorporate TAG in the milk during lactation. As secondary changes, we observe increased TAG levels in blood stream and in mammary gland. These changes might be secondary to the effect of DEX on CIDEA expression. **Financial Support**: Fapesp, Capes, CNPq *Approved by Ethics Committee of University of Campinas, Nº 4221-1/2016

07.006 Hyperlipidic diet establishes a rat model of erectile dysfunction: mechanisms underlying the endothelial damage. Souza ILL[1], Barros BC[2], Oliveira GA[2], Vasconcelos LHC[1], Silva MCC[1], Andrade LFLI[3], Cavalcante FA[1,4], Silva BA[1,5] UFPB

Introduction: Obesity is characterized by an excessive increase in body mass, leading to endothelial damage that may favor the development of diseases, such as erectile dysfunction (ED) (1). ED is defined as the constant inability to achieve or maintain a penile erection and reaches 30-56% of men worldwide (2). Recently, we showed that rats fed with hyperlipidic diet present impaired erectile function due to endothelial dysfunction (3). Thus, we aimed to characterize the mechanisms underlying the impairment of endothelium-dependent relaxation in rat corpus cavernosum. **Methods**: Wistar rats (8 weeks of age) were randomly divided into control group (CG), that received a standard diet, and obese group (OG), fed with a hyperlipidic diet during 8 weeks. Then, segments of corpus cavernosum were suspended in organ baths under appropriate conditions and isometric contractions were registered by force transducer. **Results** were expressed as mean and standard error of mean and analyzed by one way ANOVA followed by Bonferroni's posttest (n=5). **Results**: In CG, cumulative concentration-response curves to phenylephrine (PE) 10^{-8}-10^{-3} M (E_{max} = 100%; pD_2 = 5.5±0.06) were shifted to the left in a non-parallel manner with increase in E_{max} and pD_2, in the presence of L-NAME 10^{-4} M, an inhibitor of nitric oxide synthase (E_{max} = 195.9±16.7%; pD_2 = 5.8±0.1); as expected, nitric oxide (NO) impairs corpus cavernosum contraction. However, cumulative curves to PE were not altered in the presence of indomethacin 10^{-5} M, a cyclo-oxygenase inhibitor (E_{max} = 122.9±10.1%; pD_2 = 5.7±0.07), suggesting no involvement of prostanoids on penile detumescence. Meanwhile, in OG, cumulative curves to PE (E_{max} = 147.5±11.2%; pD_2 = 4.8±0.06) did not differ from that obtained in the presence of L-NAME (E_{max} = 119.3±7.6%; pD_2 = 4.9±0.1), suggesting endothelial damage due to reduce NO bioavailability. In addition, PE efficacy was reduced in the presence of indomethacin (E_{max} = 94.9±7.2%; pD_2 = 4.9±0.1), indicating an increase in contractile prostaglandins levels as a consequence of endothelial damage. Additionally, acetylcholine (ACh) 10^{-11}-10^{-4} M relaxed in a concentration-dependent manner the corpus cavernosum of both CG and OG (pD_2 = 7.6±0.1 and 7.0±0.05, respectively). However, ACh potency was 13 and 23-fold increased in the presence of tempol 10^{-3} M, a superoxide dismutase mimetic (pD2 = 8.8±0.1 and 8.0±0.3, respectively), and both 19-fold increased in the presence of apocynin 10^{-4} M, an inhibitor of NADPH oxidase (pD_2 = 8.9±0.1 and 8.5±0.2, respectively). Thus, the superoxide anion ($O_2^{\bullet}$) seems to be involved in endothelial damage development in obese rats. Conclusion: We establish a rat model of ED triggered by a low cost hyperlipidic diet consumption and characterized by endothelial dysfunction associated with decreased NO bioavailability, increased contractile prostaglandins production and $O_2^{\bullet}$ presence. Thus, providing a model for advances in sexual dysfunction field and drug discovery for ED treatment. **Financial support**: CNPq, CAPES, PPgPNSB/UFPB. Research approval: Ethical Committee on Animal Use/UFPB (0201/14). Reference: 1. Santos, Rev. Soc. Bras. Clin. Med., v. 10, p. 384, 2012 2. Costa, Drugs, v. 72, p. 2243, 2012 3. Barros, 2015, XXIII ENIC, João Pessoa/PB

07.007 ALPHA-1 adrenoceptors in an experimental model of epididymitis in rats. Mueller A[1,2], Silva EJR[1], Pupo AS[1] [1]IBB-Unesp-Botucatu – Farmacologia, [2]UFMT

Introduction: The epididymis is an organ of the male reproductive system responsible for sperm maturation, transport, storage and protection prior to ejaculation. The distal cauda epididymis (CE) is densely innervated by sympathetic postganglionic neurons that release noradrenaline (NA) to contract this tissue through alpha-1 adrenoceptors (alpha-1-ARs) activation during ejaculation (Ventura & Pennefather, BJP, 102, 540, 1991). Acute epididymitis is a highly prevalent inflammatory disease of the male urogenital tract, commonly caused by ascending bacteria to the epididymis via urethra. Epididymitis may induce asthenozoospermia leading to infertility in up to 40% of cases. This inflammatory condition has been associated to damage of CE smooth muscle (Stammler, Hum Reprod, v. 30, p. 1557, 2015). We hypothesize that changes in the CE sympathetic innervation affect the contractility of the CE, playing a role in the impairment of semen parameters induced by epididymitis. Thus, the aim of this study was to investigate the alpha-1-ARs in CE in an experimental model of epididymitis in rats induced by the injection of LPS from *E. coli* into the lumen of the vas deferens. **Methods**: adult male Wistar rats (90-110 days-old) were anesthetized with ketamine:xilazine (10mg and 100mg/kg) and intraductal retrograde injection of 25uL (25ug) of ultrapure LPS from *E. coli* O55:B55 or 25uL of sterile saline solution was performed to induce inflammation in the cauda epididymis. Rats were killed by decapitation after 6h (LPS_6h; Sal_6h) or 24h (LPS_24h; Sal_24h) of treatment and both epididymis were isolated and mounted in 10mL organ baths to record isometric tension or fixed in Bouin's solution, embedded in paraffin, and transverse sections were stained with H&E for histological analysis. Cumulative concentration-response curves to NA were obtained in the absence and presence of prazosin (alpha-1-ARs antagonist). All the concentration-response curves were performed in the presence of a cocktail of inhibitors to allow evaluation of antagonist potency (pA_2). Agonist potency was expressed as pD_2. **Results**: morphological analysis showed marked neutrophil infiltration in CE stroma of LPS_6h group, confirming the inflammatory reaction induced by LPS, as previously observed. NA induced concentration-dependent contractions of CE of all groups which were antagonized by prazosin with similar potencies (pA_2 □ 9.0) showing alpha-1-AR participation. However, NA was approximately 6-fold more potent in LPS_6h (pD_2=7.26□0.08, n=6) than in Sal_6h (pD_2=6.49□0.07, n=6, $p<0.05$), whereas there was no difference between the potency of NA in LPS_24h (6.72□0.16, n=8) and Sal_24h (6.81□0.14, n=6). **Conclusion**: LPS-induced inflammation increased the potency of NA at the alpha-1-ARs of the epididymis after 6h, indicating changes in the cauda epididymis responses to sympathetic stimulation. It will be important to investigate the effects of LPS-induced inflammation on the expression of alpha-1-AR subtypes in the CE to further determine subtype(s) involved in the contraction induced by NA and its impact on male fertility after inflammatory events. **Financial support**: Fapesp (2015/08227-0) and CNPq (479546/2013-4) Local Ethics Committee for the Use of Experimental Animals: 749-CEUA

07.008 Fetal dexamethasone exposure increased hepatic AKT2 and impaired glucose and lipid metabolism in fasted rats. Teixeira CJ[1], Murata G[2], Pantaleão LC[2], Vieira JC[2], Santos-Silva JC[2], Payolla TB[2], Mesquita FPN[1], Souza DN[1], Guimarães DED[2], Gomes PRL[2], Anhê GF[1], Bordin S[2] [1]FCM-Unicamp – Farmacologia, [2]ICB-USP – Fisiologia e Biofísica

Introduction: Proper fetal development is particularly important for establishing the lifelong health of an individual. The administration of dexamethasone (Dex) to pregnant rats results in low birth weight offspring that develop hypertension, glucose intolerance, insulin resistance, and an overactive HPA axis in later life. We have recently found that the offspring of Dex-treated dams presented the metabolic features (insulin resistance and glucose intolerance) under physiological fasting (12h-fasting). However, under a metabolic stress induced by prolonged fasting (60h-fasting), we found a severe impairment of glucose production, without any changes in insulin and glucagon levels. The molecular mechanism of this response is not yet understood, but we hypothesized that a non-canonical insulin signaling pathway takes place. The aim of this study was to investigate the activation of the non-canonical insulin signaling in the liver of adult rats exposed to Dex in fetal life. **Methods:** Nulliparous Wistar rats at 8 weeks were untreated (CTL) or treated with dexamethasone (0.1 mg/kg/day; Dex) diluted in the drinking water from the 14^{th} to the 19^{th} day of pregnancy. Pups were weaned at the 21^{o} day of life and fed with standard chow throughout. Male rats were subjected to a 60h-fasting prior the collection of the samples. Blood was used for biochemical analysis (TAG, NEFA, cholesterol) using commercial kits. Liver samples were used for immunobloting, biochemical analysis and the expression of mRNA and microRNA by qPCR. **Results:** Dex rats had increased levels of hepatic TAG, NEFA and cholesterol, without any difference in serum levels. The hepatic expression of Srebp-1c, a transcription factor that regulates fatty acid and lipid production, was also increased. Immunoblotting analysis showed increased levels of AKT2, Raptor, phospho-FoxO1, and phospho-AKT, and reduced levels of PGC1-a and phospho-CREB. In order to investigate the mechanism underlying the upregulation of AKT2 expression, we performed an in silico analysis of the rat AKT2 gene and found 3 potentials microRNA. The expression of miR-34a and miR-34c, but not miR-449a, was downregulated in the liver of Dex rats. **Conclusion:** Our results indicate that the metabolic imprinting induced by fetal Dex exposure deregulates AKT2-FoxO1 cascade and impairs hepatic metabolism, contributing to the development of steatosis and abnormal glucose production in starved rats. AKT2 overexpression is likely to result of an epigenetic regulation mediated by the downregulation of miR-34a and miR-34c. **Financial Support:** FAPESP, CNPq, CAPES **Research Approval:** 100/14 ICB-USP

07.009 Expression and Immunolocalization of the antimicrobial β-Defensin 1 in the mouse epididymis. Freitas GA[1], Scavone C[2], Avellar MCW[1] [1]Unifesp-EPM – Pharmacology, [2]ICB-USP – Pharmacology

Introduction: β-defensins are small cysteine-rich cationic proteins with pleiotropic actions, which include antimicrobial activity and immunomodulatory properties. Several β-defensins are constitutively expressed in the postnatal and adult epididymis from different species. A gradient of region- and cell-specific expression pattern of these proteins is observed in the epithelium of the adult epididymis. Their secretion into the luminal fluid and binding to spermatozoa indicate their involvement in reproduction-specific tasks. Recently we showed that a subset of six β-defensins (β-defensin 1, Defb1; Defb2, Defb12, Defb22, Spag11c and Spag11e) are differentially expressed at mRNA level during the rat Wolffian duct development, the embryonic precursor of the epididymis, contrasting with their constitutive expression in the adult rat epididymis. Aim: In the present study we have evaluated the expression and immunolocalization of one of these β-defensins, DEFB1, in the adult mouse epididymis. **Methods**: Adult Swiss mice (60 days old; N=4) were used. The epididymides were collected, dissected and divided into four regions: initial segment, caput, corpus and cauda. Tissues were either used for total RNA extraction and or frozen for obtention of cryosections. Semi-quantitative RT-PCRs were performed using primers against Defb1 and Hprt1, this latter used as a housekeeping gene. Immunosfluorescence studies were performed using antibodies against DEFB1, SPAG11C and the neuronal marker MAP1B (microtubule-associated protein 1B). **Results**: Transcripts for Defb1 were detected in all mouse epididymal regions analyzed. At protein level, DEFB1 was immunodetected throughout the epididymis in the cytoplasm and, in some cases, in the nuclei of epithelial and non-epithelial cells, such as interstitial and smooth muscle cells surrounding the epididymal duct and the blood vessels. Interestingly, DEFB1-positive immunostaining was also found in association with MAP1B-postitive nerve fibers localized along the entire epididymis, but mainly in the cauda region. No DEFB1 immunostaining was observed in the lumen of the epididymal tubules, indicating no association with luminal spermatozoa. Spermatozoa-positive staining against the β-defensin SPAG11C, used here as positive control. Fewer MAP1B-positive nerve fibers were observed in immunofluorescence studies against SPAG11C when compared to the studies with the antibody against DEFB1. No staining was observed when immunofluorescent assays were performed in the absence of the primary antibodies, used as negative controls. **Conclusions**: Collectively the results corroborate to the understanding of the role of β-defensins in the adult epididymis. The novelty of the data relies on the differential detection of β-defensins associated with nerve fibers in the epididymis, broadening the potential biological roles of this protein family in epididymal biology. **Financial support**: CAPES, CNPq, FAPESP (#2014/19378-6). Ethics Approval: CEUA UNIFESP-EPM #7991170915/2015.

07.010 Cilostazol causes inhibition of contraction in the iliac artery and potentiates the cGMP pathway. Justo AFO[1], Calmasini FB[1], Alexandre EC[1], Campos RM[1], De Nucci G[1 1]FCM-Unicamp – Farmacologia

Introduction: Lower urinary tract symptoms (LUTS), characterized by, urinary incontinence, urinary frequency, urgency, nocturia, bladder pain and voiding difficulties causes a large decrease in the population's quality of life, being more prevalent in the elderly. The main of LUTS causes are bladder outlet obstruction, ischemia and sympathetic hyperactivity. Abdominal aorta and its branches, especially the iliac arteries, are particularly vulnerable to the atherosclerotic lesions and occlusion during aging. Iliac artery is responsible for the supply of the genitourinary tract, such as, bladder, urethra, prostate and penis. Cilostazol is a phosphodiesterase 3 inhibitor and acts inhibiting the hydrolysis of cGMP and cAMP. Aims:The purpose of this study is to investigate the effects of cilostazol in iliac artery isolated from rats. **Methods**: Concentration response curves to phenylephrine (100pM to 300µM) were constructed in iliac arteries in presence and absence of pre-treatment with cilostazol (10µM). In other set of experiments, iliac arteries were pre-contracted with phenylephrine (1µM) and when obtained a sustained contraction, a single concentration of cilostazol (10µM), forskolin (30nM), sodium nitroprusside (30nM), forskolin (30nM) + cilostazol (10µM) and sodium nitroprusside (30nM) + cilostazol (10µM) were addicted. **Results**: Cilostazol reduced 56,6% the maximal response (from 28,82mN±3,42 to 12,5mN±2,20) and the potency (shift of 5.2) of phenylephrine-induced iliac artery contractions. It was performed single doses of SNP(30nM) and forskolin (30nM) in precontracted artery by phenylephrine, 63,67% and 52,96%, respectively; Cilostazol 10 µM was added and synergistically increased the relaxation evoked by forskolin 84,35% and SNP 82,61%. Fosrkolin and SNP promoted relaxation in iliac arteries precontracted with phenylephrine (52.96%and 63.67% respectively), showing a inhibitory effect on iliac artery contraction. Conclusion: Cilostazol induces relaxation on iliac artery and potentiates the relaxation induced by nitric-oxide (NO)-cGMP pathway. Financial Support: FAPESP (2015/15583-7) (2016/09539-8) CEUA 4120-1. **Reference**: ANDERSSON KE; NOMIYA M; SAWADA N; YAMAGUCHI. Pharmacological treatment of chronic pelvic ischemia. Therapeutic Advances in Urology.2014:6(3): 105-114 MATSUMOTO S; WATANABE M; HASHIZUME K; WADA N; et. al. Effects of Chronic Treatment With Cilostazol, a Phosphodiesterase 3 inhibitor, on female Rat Bladder in a Partial Bladder outlet Obstruction Model. Urology 83 (3), 2014: 7-11.

07.011 Different β-defensins display contrasting gene expression and cellular distribution patterns during rat Wolffian duct morphogenesis. Ferreira LGA[1], Ribeiro CM[1], Hinton BT[2], Avellar MCW[1] [1]Unifesp-EPM – Farmacologia, [2]University of Virginia School of Medicine

Introduction: β-defensins, primarily known as antimicrobial proteins, are abundantly expressed in the postnatal and adult epididymis from several species. In addition, studies have indicated their involvement in reproduction-specific tasks. Interestingly, we showed that the β-defensin SPAG11C (sperm-associated antigen 11C) is present in the rat Wolffian duct (WD), the embryonic precursor of the epididymis, and acts on WD morphogenesis, an androgen-dependent event. Preliminary RT-PCR studies from our group revealed differential expression pattern between Spag11c and other β-defensins in the WD, contrasting with their constitutive expression in the adult epididymis. Would other β-defensins be involved in epididymal morphogenesis? To answer this question, we investigated the gene expression and immunodistribution of the β-defensins DEFB2 (defensin beta 2) and SPAG11E during WD development. **Methods**: WDs from male Wistar rats at embryonic days (e) e12.5, e14.5, e17.5 and e20.5 (n=3-5 embryos/age) were collected to analyze β-defensin mRNA levels by RT-qPCR assays. Caput epididymides from 120 days-old adult rats were used as positive control. Testosterone levels in plasma collected from male embryos at e17.5 and e20.5 (n=7 embryos/age) were determined by ELISA. Immunohistochemical study was performed on paraffin-embedded tissue sections from isolated adult epididymides (n=4 animals) and whole fetuses at e20.5 (n=3 embryos). DEFB2 and SPAG11E mRNA expression pattern and cellular distribution were compared to that previously described for SPAG11C. **Results**: Transcripts for all β-defensins tested were detected in high abundance in the adult caput epididymis, as we expected. Furthermore, immunoreaction for all of them was also observed in the adult epididymis. Distinctly, Spag11c mRNA was detected in WDs between e12.5-e20.5, while the presence of Defb2 mRNA was only observed in WDs at e20.5. Spag11e transcript was not readily detected in WDs at any time-point analyzed. Spag11c mRNA levels decreased (2.4-fold) and Defb2 mRNA levels increased (2.4-fold) in WDs between e17.5-e20.5, a period when the fetal plasma testosterone levels rise (4.69 ± 0.40 nM vs 7.15 ± 0.40 nM; mean ± SEM) and the androgen-induced differentiation of WD into epididymis occurs in the rat. In terms of protein localization, SPAG11C was immunodetected mainly in mesenchymal cells of the WD at e20.5, contrasting to the DEFB2 immunostaining observed in mesenchymal and epithelial cells at this time-point. Reflecting the transcript level, SPAG11E was not immunodetected in the WD at e20.5. **Conclusions**: The novelty of our data is the contrasting mRNA expression profile and immunodistribution of β-defensins during androgen-induced development of the WD. Our results may reflect specific regulatory mechanisms and/or distinct biological roles for these proteins, broadening their relevance to epididymal function and male fertility. **Financial support**: CNPq/Science Without Borders (401932/2013-3, 1050066/2016-3, 101550/2016-2), NIH−NICHD #069654, FAPESP #2016/00164-1. Ethics Approval: CEUA Unifesp-EPM N. 1776201213.

07.012 Impact of LPS- and LTA-induced epididymitis on sperm parameters in rats. Silva AAS[1], Silva GC[1], Ribeiro CM[2], Avellar MCW[2], Silva EJR[1] [1]IBB-Unesp-Botucatu – Farmacologia, [2]Unifesp-EPM – Farmacologia

Introduction: Infection and inflammation of the male reproductive tract are relevant etiological factors of infertility. The epididymis, an organ with crucial roles on sperm maturation, is highly susceptible to ascending canalicular infections through the urethra leading to epididymitis. This disease is the most common cause of scrotal pain and may induce permanent or transient infertility. Bacteria such as *Escherichia coli (*Gram negative) and *Enterococcus faecalis (*Gram positive) are common causative pathogens of epididymitis. Despite the negative impact of bacterial epididymitis on reproduction, its possible pathogen-specific fertility outcomes are yet to be understood. Here, we investigated the effects of epididymitis induced by bacterial-derived products LPS from *E. coli* or LTA from *E. faecalis* on testicular and epididymal sperm parameters in rats. **Methods**: Wistar rats (90 days, n=5/group) were anesthetized with ketamine/xylazin (100 and 10 mg/kg, i.p.) and submitted to an experimental model of epididymitis based on the retrograde injection of saline (control), LPS from E. coli (25 µg) or LTA from E. faecalis (125 µg) into the lumen of vas deferens, thus mimicking an infection of the epididymis. Rats were euthanized 1, 7 and 15 days after treatment; their testes and epididymides (divided into caput/corpus and cauda) were weighed and processed for sperm count. We evaluated the following parameters: number of homogenization-resistant spermatids and daily sperm production (DSP), in the testis; and number of spermatozoa and sperm transit time, in the cauda epididymis. Epididymal sperm count was performed in the 7-day treated groups only. Data were analyzed by ANOVA followed by Bonferroni's test ($p<0.05$ was considered significant). **Results:** Intravasal LPS or LTA treatment did not change testis and epididymis weight, as well as the absolute number (10^6 x cells/organ) of homogenization-resistant spermatids and DSP in any experimental group. The relative number (10^6 x cells/g of organ) of homogenization-resistant spermatids and DSP, however, was significantly increased by 13.4 and 13.2%, respectively, in the 7-day LPS group in comparison to its control group. In the cauda epididymis, sperm transit time and both the absolute and relative sperm counts were significantly reduced by 40.4, 36.1, and 34.1%, respectively, in the 7-day LPS group in comparison to its control group. No changes in cauda epididymal sperm parameters were observed in the 7-day LTA group. **Conclusions**: Our results showed that epididymitis induced by LPS or LTA differentially affected sperm parameters in rats. The impact of LPS, but not LTA, on sperm count and transit time in the cauda epididymis suggested a differential sensitivity of this region to Gram-positive and Gram-negative bacteria, which may contribute to distinct reproductive outcomes based on the type of infection. **Financial Support:** This study was supported by Science without Borders Program (CSF/CNPq), CNPq, Fapesp, and PROPe-UNESP. This study was approved by the Research Ethics Committee from IBB-UNESP (process #629) and UNIFESP-EPM (process #0310/12).

07.013 Use of gene expression as a marker of efficacy in the pharmacological treatment of levothyroxine in hypothyroid individuals. Silva VA, Almeida RJ, Teixeira PVL, Silva LM, Pesquero JB, Camacho CP Uninove – Biophysics.

The primary hypothyroidism is characterized by dysfunction of the thyroid gland, one of the most common endocrine disease and affects approximately 16% of the population. The available therapy is treatment with levothyroxine (LT4), a synthetic conformation of the hormone thyroxine. Although this therapy is relatively simple and inexpensive, often therapeutic targets are not met. Multiple factors can influence this endpoint and possibly generate different pharmacokinetic changes, starting from a broad perspective; deiodination can be seen as a model that can lead to the inactivation of hormones in specific tissues. Since the absorption of levothyroxine mechanisms are not fully understood, the goal of the study is to create a gene expression panel to mark the therapeutic response. **Methods**: This research was approved by the IRB of our institution (IRB number: 665331 and 679727). We evaluated 320 individuals and selected eight patients with levothyroxine therapy to RNA-Seq Transcriptome analysis. They are divided into two groups. The first group has four hypothyroid patients with Thyroid Stimulant Hormone (TSH) between 0.5 mIU/L and 2.0 mIU/L (In LT4 therapy for a mean time of seven years) and four patients with TSH between 4.0 mIU/L and 20.0 mIU/L (In LT4 therapy a mean time six and a half years). Venous blood samples were collected (5 mL) in the morning and stored in PAXgene RNA tubes. The RNA extraction was realized with PAXgene RNA extraction kit (Qiagen). The transcriptome library was created in an NGS platform, Ion Proton System, following the kit protocols of Ion AmpliSeq Gene human transcriptome (Thermo Fisher Scientific Manufacturer). The computational analysis of data was performed in 0.99.491 rstudio software, Package Edger 3.12.0 of Bioconductor (Robinson MD, DJ McCarthy and Smyth GK, 2010). The Spearman test was used to correlate the reads and TSH values through the IBM SPSS version 20 software. **Results**: The differential expression analysis revealed 179 with an increase and 174 with decrease in the expression, where 289 were mRNA and 64 non-coding RNA. Of these, 48 genes correlated with TSH, and 2 genes showed a correlation superior to 0.9 (*SHISA4* and *NCS1*). Conclusion: We constructed a panel able to identify patients with an inadequate response to treatment with levothyroxine. The panel can be useful in future studies as a tool to assess more accurately the levothyroxine response. FAPESP – Nº 2014/04193-0 License of the Ethics Committee: CEP665331 and CEP679727

07.014 Effectiveness and clinical inertia in the management of type 2 diabetes mellitus in patients in Colombia. Machado-Duque M, Machado-Alba J, Ramirez-Riveros C

Introduction: Determine the effectiveness of antidiabetic therapy and frequency of clinical inertia in the management of type 2 diabetes mellitus (DM-2) in Colombia - 2015. **Methods**: Retrospective cross-sectional study with follow-up of patients receiving treatment for diabetes, treated at medical consultation during a year in 23 cities. Effectiveness was established (HbA1c <7%) and clinical inertia was considered when no modification of therapy occurred despite not achieving control goals. Sociodemographic, clinical, pharmacological, therapeutic monitoring and clinical inertia were evaluated. Multivariate analyzes were performed. **Results:** A total of 363 patients DM-2 were included, with a mean age of 62 ± 12.2 years and 53.4% were women. A total of 1016 consultations were evaluated and the therapy was effective at the end of follow-up in 57.9% of cases, in those cases with HbA1c control not achieved, clinical inertia was found in 56.8%. The drug most frequently prescribed was metformin (84.0%), followed by glibenclamide (23.4%) and insulin glargine (20.7%). 43.8% of patients were treated with 2 antidiabetics. Logistic regression showed that to be controlled in the first follow-up consultation was a protective factor against clinical inertia in the following meetings (Odds Ratio: 0.09; 95% Confidence Interval: 0.04 to 0.17; p <0.001). **Conclusions:** Is the first description of clinical inertia in DM-2 conducted in Colombia, demonstrating the possible negative impact on the control and treatment of the disease, despite this, the effectiveness of antidiabetic therapy has increased compared to previous measurements. **Bioethics:** The investigation was approved by the Bioethics Committee of the Universidad Tecnologica de Pereira, respecting the confidentiality principles of the Declaration of Helsinki. **Financial Support:** Universidad Tecnológica de Pereira and Audifarma S.A **References:** Philips LS. Ann Intern Med. 2001;135: 825-34. Machado-Alba JE. Rev Panam Salud Publica. 2009;26(6):529-35. Machado-Duque ME. J Am Soc Hypertens. 2015 Nov;9(11):878-84. Gonzalez-Clemente JM. Med Clin (Barc). 2014;142(11):478-84.

07.015 Histomorphometric evaluation of corpus cavernosum in spontaneously hypertensive rats with 5-α-reductase inhibitors treatment. da Silva MHA, de Souza DB, Costa WS, Sampaio FJB UERJ – Fisiopatologia e Ciências Cirúrgicas

Introduction: The experimental model of spontaneously hypertensive rats (SHR) is also considered the best model for benign prostatic hyperplasia (BPH) and lower urinary tract syndrome studies. BPH affects 50% of men between with 50-60 years and the first-line treatment is based on 5-α-reductase inhibitors. The 5-α-reductase is an enzyme responsible for converting testosterone to dihydrotestosterone, which is the most hormonal active form. Recent studies reported that the use of these medications may lead to erectile dysfunction (ED), probably because of low dihydrotestosterone acting in penile tissue. However, to date there are no scientific studies that investigates the corpora cavernous morphology after 5-α-reductase inhibitors treatment. **Methodology:** We use 40 adult male rats which were divided into 4 groups (n=10 per group): Group Wistar Kyoto (WKY); SHR (H); SHR treated with dutasteride (HD, 0.5 mg/Kg/day); Group SHR treated with finasteride (HFN, 5mg/Kg/day). The animals were killed with sodium thiopental after 40 days of experiment. At euthanasia, the penis was dissected and the following histomorphometrical analysis were performed: total area of the penis, cavernous area with the tunica albuginea, cavernous area without the tunica albuginea, surface density (Sv) of connective tissue, sinusoid, and smooth muscle. Data were analyzed by unpaired ANOVA, considering $p<0.05$. **Results:** There was a reduction of the total area of the penis in H (-12.17%), HD (-13.16%) and HFN (-14.21%) groups, when compared of the WKY group. The cavernous area with the tunica albuginea was reduced in H (-12.21%), HD (-12.21%) and HFN (-13.55%) groups, when compared of the WKY group. When analyzing the cavernous area without the tunica albuginea there was a reduction in HFN (-24.98%) groups, when compared of the WKY group. Regarding the Sv[connective tissue] there was an increase in H (+13.72%), HD (+28.36%) and HFN (+15.79%) groups when compared to WKY group. There was an increase of Sv[connective tissue] in HD (+12.88%) when compared to group H, and a reduction in HFN (-9.80%) when compared to the HD group. Regarding the Sv[sinusoid] there was a reduction in H (-33.68%) and HD (-47.26%) when compared with the WKY group. An increase was observed in HFN (+61.07%) when compared to HD. The Sv[smooth muscle] was reduced in HD (-26.72%) and HFN (-30.80%) when compared with the WKY group. When comparing the H group with HD and HFN, a reduction of 29.24 and 33.19% was observed, respectively. **Conclusion:** The rodent model of BPH shows some cavernous morphometrical alterations that may correlates with the ED seen in clinical settings. The animals receiving 5-α-reductase inhibitors treatment showed even worse cavernous morphology, with reduction in penile area and smooth muscle and increase in connective tissue. This may be correlated with penile fibrosis as seen in men with ED. Among the used drugs, the dutasteride was the one that lead to worst alterations. **Source of Funding:** CAPES, CNPq and FAPERJ. The protocols used were approved by CEUA 051/2012, UERJ.

07.016 N-Acetylcystein action on biomarkers of oxidative stress in the myocardium of diabetic rats. Kaga AK, Barbanera PO, Carmo NOL, Silva DF, Queiroz PM, Rosa LRO, Fernandes AAH IBB-Unesp-Botucatu – Química e Bioquímica

Introduction: Diabetes mellitus (DM) is characterized by chronic hyperglycemia due to the deficient production/action of insulin. The hyperglycemia generates reactive oxygen species, which promotes damage in important tissues [1] such as cardiac [2]. N-acetylcysteine (NAC) has antioxidant potential to be precursor of L cysteine and reduced glutathione (GSH) in the cells [3]. Thus it can attenuate reduce the oxidative stress and control DM. **Objectives:** Evaluate the effect of NAC on biomarkers of oxidative stress in cardiac tissue of rats with diabetes mellitus type 1 (DM1). **Methods:** 32 male wistar rats (±220g; 50 days old) were distributed in groups (*n*=8): G_1: normal rats; G_2: normal rats treated with NAC, G_3: diabetic rats; G_4: diabetic rats treated with NAC. The rats received water and a rodent chow *ad libitum*. Experimental DM1 was induced by streptozotocin ($60mg^{-1}Kg$ body weight, single dose, i.p.). After 48 hours, animals with glycemic level above of 250 mg/dL were considered diabetic. The animals received NAC ($25\ mg^{-1}kg^{-1}day$) by gavage for 35 days. After the experimental period the animals were anesthetized and euthanized. Serum and cardiac samples (±100mg) were collected. Statistical analysis was performed using One-way ANOVA, and the means were compared using the Tukey test with $p<0.01$. **Results**: NAC reduced glycemia in diabetic rats ($p<0.01$), but without reaching the values obtained for G1 (G_1: 92,71±14,41; G_2: 98,10±14,07; G_3: 351,04±27,94; G_4: 128,84±12,87 mg/dL. The cardiac concentration both total gluthatione (GS) and reduced (GSH) decreased ($p<0.01$) in G_3, when compared to the other groups (GS: G_1:102,68±14,23; G_2:98,27±8,94; G_3:77,40±8,96; G_4:101,54±8,86 nmol/mg tec; GSH: G_1:72,41±7,44; G_2:86,60±7,14; G_3:51,84±9,46; G_4:84,44±9,53 nmol/mg tec). The G3 group exhibited higher myocardial lipid hydroperoxide, and NAC decreased ($p<0.01$) (G_1:199,51±40,75; G_2:159,06±34,92; G_3:255,99±53,31; G_4:193,03±45,05 nmol/g tec). Untreated diabetic rats showed decreased ($p<0.01$) of total proteins in myocardium, while administration of NAC caused increases (G_1:33,70±1,40; G_2:30,47±0,98; G_3:25,37±1,08; G_4:31,01±1,43 mg/100mg tec). **Conclusion:** NAC controlled the hyperglycemia and normalized the biomarkers of oxidative stress in myocardium under diabetic conditions. **References:** [1] ROLO, A.P. Toxicol Appl Pharmacol. V.212, p.167, 2006. [2] BATTIPROLU, P.K. Life sci. v.92, p.609, 2013. [3] SADOWSKA, A.M. Pulm. Pharmacol. Ther. v.20, p.9, 2007. **Financial Support:** CAPES; FAPESP **Animal Research Ethical Committee:** Approved (No. 706) by the Ethics Committee on the Use of Animals (CEUA) at the Institute of Biological Sciences, University of São Paulo State (UNESP).

07.017 Estrogen receptors ESR2 play a role in the differentiation of Sertoli cells from 20-day-old rats. Macheroni C, Nascimento AR, Lucas TFG, Porto CS – Unifesp-EPM – Farmacologia

Introduction: Sertoli cells play a key role in the control of germ cell development. Androgen, FSH, thyroid hormone, and growth factors are classically known mediators of Sertoli cell proliferation and differentiation (Lucas et al., Spermatogenesis 4, e28138, 2014a). Our laboratory has shown the presence of the classic estrogen receptors ESR1 (ERalpha) and ESR2 (ERbeta) in cultured Sertoli cells obtained from 15-day-old rats. Furthermore, the complex E2/ESR1 modulates proteins related with Sertoli cell proliferation Cyclin D1, and this effect was dependent on ERK1/2 and NF-kB activation. E2/ESR2 regulates proteins related with cell cycle exit and differentiation $p27^{kip1}$, GATA-1 and DMRT1, in a PI3K and CREB-dependent manner (Lucas et al., Mol Cell Endocrinol 382:84, 2014b). Analyzing the expression of ESR1 and ESR2 in different stages of development of Sertoli cells was observed that the ESR1/ESR2 ratio decreased with age, and this ratio seems to be important to determine the end of cell proliferation and the start of cell differentiation. In fact, in Sertoli cells from 15-day-old rats, the ESR1/ESR2 ratio favors the effect of ESR1 and the activation of this receptor induces Sertoli cell proliferation. In cultured cells from 20-day-old rats, the expression of ESR2 is higher than ESR1 (Lucas et al., 2014b), whether ESR2 plays a role in differentiation remain to be explored. Thus, the aim of the present study was to investigate the role of ESR2 on the regulation of proteins involved with proliferation and differentiation of Sertoli cells from 20-day-old rats. **Methods**: The experimental procedures were approved by the Research Ethical Committee from EPM-UNIFESP (3723240815/2015). Primary culture of Sertoli cells was obtained from 20-day-old rats, as previously described (Lucas et al., 2014b). Cells were treated with E2 (0.1 nM), ESR1-selective agonist PPT (10 nM) or ESR2-selective agonist DPN (10 nM) for 24 hours and Western Blot for detection of Cyclin D1, p^{27kip1} and actin were performed as previously described (Lucas et al., 2014b). **Results and Conclusions**: In Sertoli cells with development of the animals (15 and 20-day-old rats), the differential expression of Cyclin D1 decreased 2-fold, while $p27^{kip1}$ increased 3-fold. In Sertoli cells from 20-day-old, the treatment with PPT or DPN did not have any effect on Cyclin D1 expression. On the other hand, the expression of $p27^{kip1}$ increased 2-fold with DPN. These results suggest the involvement of ESR2 in Sertoli cell differentiation. The present study reinforces the important role of estrogen for normal testis development and homeostasis, and may direct further studies to better understand the causes of male infertility. Financial Support: FAPESP, CNPq, CAPES.

07.018 Prenatal dexamethasone treatment disrupts the physiological UPR-induced burst of apoptosis in islets of lactating rats. Souza DN[1], Santos-Silva JC[2], Silva PMR[1], Ferreira DS[1], Sollon CS[1], Mesquita FPN[1], Teixeira CJ[1], Gomes PR[2], Anhê GF[1], Bordin S[2] [1]FCM-Unicamp – Farmacologia, [2]ICB-USP – Biofísica e Fisiologia

Introduction: A hallmark of the metabolic adaptation to pregnancy is the increased maternal insulin secretion in the mid-to-late pregnancy. However, in order to avoid severe postnatal hypoglycemia, the pancreatic beta cell gain-of-function must be precisely reverted. We have previously demonstrated that the activation of Unfolded Protein Response (UPR) mediates the physiological and transient increase in maternal beta cell apoptosis after delivery. UPR reduced AKT phosphorylation through ATF4/CHOP-induced TRB3 expression. That process is likely to be essential to beta cell renewing, driving the endocrine pancreas to the non-pregnant state just after delivery. On the other hand, glucocorticoid (GC) excess in late pregnancy results in progressive and permanent glucose intolerance, mainly due to a premature senescence of beta cell. Thus, we hypothesized that the excess of GC could impair the physiological burst of apoptosis after delivery. The aim of this work is to evaluate the rate of apoptosis and the activation of UPR in pancreatic islets of early lactating rats treated with dexamethasone during pregnancy. **Methods**: Nulliparous Wistar rats were treated with dexamethasone (0.1mg/kg/day; DEX) diluted in the drinking water from the 15th to the 19th day of pregnancy. Pancreatic islets were isolated by collagenase digestion from virgin rats (CTL), and from rats at the 20th day of pregnancy (G20) and at the 3th day of lactation (L3). Isolated islets were used for standard protein extraction and immunoblotting. Also, pools of 100 islets were disrupted with a Ca2+-free medium and used to the analysis of DNA fragmentation using propidium iodide staining and fluorescence, measured by flow cytometry. **Results**: DNA fragmentation was lower in islets from L3-DEX rats as compared to islets from L3, reaching values similar to CTL group. In islets of G20 group, DNA fragmentation was not different from CTL, but G20-DEX islets also showed lower rate of apoptosis. Similarly to DNA fragmentation, protein levels of CHOP, ATF4 and TRB3 were higher in islets from L3 but not from L3-DEX. AKT phosphorylation was lower in islets from L3 as compared to CTL and L3-DEX islets. **Conclusion**: Lower levels of URP-related proteins, higher levels of AKT phosphorylation, and reduced DNA fragmentation in L3-DEX islets indicate that the UPR-induced maternal beta cell apoptosis was impaired by prenatal dexamethasone treatment. **Financial Support**: FAPESP, Capes, CNPq *Approved by Ethics Committee of University of Campinas, Nº 3505-1

07.019 The modulation of rat seminal vesicle smooth muscle by purinergic transmission.
Kiguti LRA, Campos RM, Antunes E Unicamp – Farmacologia

Introduction: Postganglionic sympathetic neurons release ATP and noradrenaline (NA) as cotransmitters to a number of urogenital smooth muscles. Released ATP contract smooth muscle via ionotropic P2X1 receptors and is metabolized sequentially to ADP, AMP and adenosine (Ado), which have their own effects by activation of a plethora of metabotropic G protein coupled receptors. Ado, the major ATP metabolite accumulated following sympathetic activation, has excitatory and inhibitory effects on neurotransmission by activation of pre- or postsynaptic Ado receptors (AdoR; i.e. A1R, A2AR, A2BR and A3R). The purinergic transmission to the rat seminal vesicles (SV) smooth muscle is less known. This study investigated the putative P2X1 and AdoR purinergic transmission in the rat SV through in vitro contraction and relaxing studies. **Methods**: All the experimental procedures were approved by the institutional committee for the use of experimental animals (CEUA: 4074-1). Adult male Wistar rats (120-180 days old) were killed and both SV were isolated to in vitro contraction recording (10ml organ baths, Krebs solution). The effects of the P2X1 antagonists suramin (30uM) and PPADS (30uM), the alpha1-adrenoceptor antagonist prazosin (0.3uM) and the muscarinic receptor antagonist atropine (0.3uM) were investigated on the SV contractions induced by electrical-field stimulation (EFS; 60V, 1ms pulse, 8-50Hz). The effects of exogenous ATP, the AdoR agonists N6-cyclopentyladenosine (CPA, A1R-selective) and NECA (non-selective) and the AdoR antagonists DPCPX (A1R-selective; 10nM) and ZM 241385 (A2A-selective; 3-30nM) were evaluated on NA (10uM)-induced SV contractions. **Results**: EFS induced tetrodotoxin-sensitive frequency-dependent SV contractions, which were biphasic at low-to-intermediate frequencies (8, 16 and 32Hz) and monophasic at high stimulation frequency (50Hz). Both phases of EFS-induced SV contractions were almost abolished by prazosin (90% reduction) at all stimulation frequencies whereas suramin and PPADS had no effect. The remaining SV contraction to EFS in the presence of prazosin was abolished by atropine. Accordingly, exogenous ATP (until 3mM) did not contract the rat SV (n=6). Interestingly, ATP (1mM) decreased by 3-fold the potency of NA in inducing rat SV contraction, an effect prevented by 100nM DPCPX (n=3). CPA inhibited the EFS-induced twitch-like SV contraction (pD_2=7.74±0.11, n=5) in a DPCPX-sensitive manner (pA_2≈8.70). The AdoR agonist NECA concentration-dependently relaxed the NA pre-contracted SV (pD_2: 6.57±0.14; Maximal relaxation: 62.43±2.45% of NA-induced contraction; n=6), an effect non-competitively antagonized by ZM 241385 (pA_2=9.01±0.13, n=4). **Discussion**: Rat SV smooth muscle contraction is modulated by A1R and A2AR receptors, with no roles for P2X1. Our results suggest that released ATP acts as a negative regulator of rat SV contraction, an effect mediated, at least in part, by AdoR activation. Whether the ATP effects on rat SV smooth muscle contraction are brought by Ado synthesis and the AdoR subtypes involved on ATP effects are under investigation. **Financial support**: FAPESP (2015/19677-6, LRAK).

07.020 Nitric oxide donor compound 3-(1,3-dioxoisoindolin-2-yl)benzyl nitrate reverses increased nitric oxide-mediated cavernosal relaxations in transgenic sickle cell mouse model of priapism. Silva FH[1], Karakus S[2], Musicki B[2], dos Santos JL[3], Costa FF[1], Burnett AL[2]
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Introduction: Sickle cell disease (SCD) patients display priapism, and dysregulated nitric oxide (NO) pathway can contribute to this conditions. However, current therapies offered for the prevention of priapism in SCD are few. The 3-(1,3-dioxoisoindolin-2-yl)benzyl nitrate (compound 4C) was synthesized through molecular hybridization of hydroxyurea and thalidomide, which displays NO-donor property. This study aimed to evaluate the effects of compound 4C on functional and molecular alterations of erectile function in SCD transgenic mice, focusing on the dysregulated NO-cGMP-PDE5 pathway in erectile tissue. **Methods:** All animal procedures were conducted in accordance with the ethical standards of the Johns Hopkins University School of Medicine Guidelines for the Care and Use of Laboratory Animals (M014M100). Wild type (WT) and $dNOS^{-/-}$ mice were treated with compound 4C (100 mmol/kg/day, 3 weeks). Corpus cavernosum tissue was dissected free, and mounted in organ baths. Mouse corpus cavernosum relaxations induced by acetylcholine (ACh), sodium nitroprusside (SNP) and electrical field stimulation (EFS) in phenylephrine (10 µM)-pre-contracted tissues were carried out in all groups. The protein expression for PDE5 in mouse penis was also evaluated. **Results:** The cumulative addition of ACh (10^{-9} to 3×10^{-6} M) to PE-contracted tissues produced concentration-dependent relaxations in all groups. The ACh potency (pEC_{50}) value was significantly higher ($P < 0.05$) in corpus cavernosum of SCD compared to WT mice, which was reversed by long-term treatment with 4C. The maximal response (E_{max}) induced by SNP was significantly higher ($P < 0.05$) in corpus cavernosum from SCD (93 ± 6 %) compared to WT mice (72 ± 5 %), which was fully restored with compound 4C treatment. Likewise, corpus cavernosum relaxations to EFS was significantly higher ($P < 0.05$) in SCD compared to WT mice, as observed at 2 and 8 Hz. Long-term treatment with compound 4C reduced ($P < 0.05$) the EFS-induced increased relaxant responses in corpus cavernosum from SCD. No significant changes after compound 4C treatment were observed in ACh-, SNP- and EFS-induced corpus cavernosum relaxations of WT mice. The protein expressions for PDE5 were significantly reduced ($P < 0.05$) in penile tissues from SCD in comparison with the WT group. Treatment with compound 4C significantly increased ($P < 0.05$) the protein levels of PDE5 in the penises from SCD, with no significant changes in WT-4C group. **Conclusion:** In conclusion, three-week therapy with NO donor compound 4C reversed the exaggerated NO-mediated cavernosal relaxations and normalized PDE5 expressions in the penises from SCD mice. NO donor compounds may constitute an additional strategy to prevent priapism in SCD. **Financial Support:** FAPESP (2013/19781-2, 2014/00984-3).

07.021 Epidermal growth factor pathway regulates androgen-induced Wolffian duct morphogenesis. Ribeiro CM[1], Hinton BT[2], Avellar MCW[1] [1]Unifesp-EPM – Farmacologia, [2]University of Virginia School of Medicine – Cell Biology

Introduction: Androgens drive the morphological changes the Wolffian duct (WD) undergoes during embryonic development to originate the epididymis, which is a highly convoluted epithelial tube that provides the luminal environment required for sperm maturation and male fertility. Androgens act indirectly in WD epithelium via mesenchyme-derived regulators by mechanisms that are still poorly understood. Growth factors are major candidates that may mediate androgen action in this morphogenetic program. Here we evaluated the role of epidermal growth factor (EGF) pathway in androgen-dependent WD morphogenesis. **Methods**: WDs were dissected from male Wistar rats at embryonic day 17.5 and used for RT-PCR or cultured for up to 96 h on cell inserts floating on serum-free medium, in the absence or presence of testosterone (1-10 nM), EGF (1.7-17 nM), PD98059 (50 µM, MEK inhibitor) and AG1478 (10-20 µM, selective inhibitor of EGF receptor kinase). Gross morphology was evaluated. **Results**: *Egf*, *Egfr* (Egf receptor) and *Etv4* (Ets Variant4; a downstream target of growth factors signaling) transcripts were detected in WDs at e17.5. EGF promoted elongation and coiling of cultured WDs in a concentration-dependent manner. A synergistic effect of testosterone and EGF was observed on morphogenesis of cultured WDs. However, the elongation and coiling pattern induced by testosterone in cultured WDs was not mimicked by the incubation of EGF alone. The total WD area was higher in EGF-treated WDs in comparison to ducts cultured with testosterone, an effect blocked by co-incubation of EGF and AG1478 or EGF and PD98059. Furthermore, blockade of either EGFR phosphorylation with AG1478 or blockade of ERK signaling with PD98059 impaired testosterone-induced WD morphogenesis. Conclusion: Our results suggest that EGF/EGFR signaling regulates androgen-dependent WD morphological differentiation. In addition, we showed that androgen action in WD morphogenesis depends on activation of EGFR and MAPK/ERK signaling. These findings highlight the importance of the cross-talk between AR and growth factor receptor signaling pathways during embryonic development to determine future reproductive disorders. **Financial support**: CNPq/CSF (401932/2013-3, 150066/2016-3), NIH−NICHD #069654. Ethics Approval: CEUA-Unifesp-EPM#1776201213.

07.022 Antidiabetics Prescription Patterns and Costs in a Group of Patients from Colombia, 2015 Gaviria-Mendoza A, Machado-Alba J, Medina-Morales D, Sanchez-Duque J Audifarma S.A. – Investigacion Farmacoepidemiológica

Introduction: Diabetes mellitus is a chronic non-communicable disease of great interest to global health by its burden of morbidity and mortality and high demand of financial resources for prevention and treatment. The prevalence of type 2 diabetes in Colombia is 4.8 to 7.3%. The objective was to determine the prescribing patterns of antidiabetic drugs and the variables associated with their use in a Colombian population. Methodology: A descriptive cross-sectional study. Through a systematized database of approximately 3.5 million people insured by the Colombian Health System (approximately 7.2% of the Colombian population), patients of both sexes and all ages treated with antidiabetic drugs continuously for at least three months were included (1 June to August 31, 2015). A database that comprised sociodemographic, pharmacological, co-medication and cost variables was designed. The data were analyzed using SPSS-23, a P-value of <0.05 was considered to be significant. **Results**: 47,532 patients were included; the mean age was 65.5 years, and 56.3% were women. 56.2% (n = 26,691) of people received monotherapy and 43.8% (n = 20,841) were prescribed with two to four antidiabetic drugs. The most prescribed medications were: metformin 81.3% (n=38,664), insulins 33.3% (15,848), sulfonylureas 21.8% (10,370) and inhibitors of dipeptidyl peptidase-4 13.7% (6,510). The mean prescribed daily dose to defined daily dose ratio was: 0.75 for metformin, 0.85 for glibenclamide and 1.30 for total insulin (the last presented high variability). 92.8% (n=44,106) of patients received concomitant treatment: antihypertensives (79.7%), lipid-lowering drugs (65.5%), antiplatelet agents (56.3%), analgesics (33.9%), antiulcer medication (33.1%), thyroid hormone (17.2%) and psychotropic drugs (14.9%). The total cost of metformin during the observation period was USD 59,036, while the costs of NPH insulin was USD 36,164 and USD 431,792 for glargine insulin. **Discussion**: In general, rational prescribing habits prevailed, with use of drugs of high therapeutic value in doses close to the defined daily dose and in combination therapy with sufficient scientific support, however, in some cases overuse of some drugs is observed (such as anti-ulcer medications). **Financial support**: This work was funded by the Universidad Tecnológica de Pereira and Audifarma S.A. The Bioethics Committee of the Universidad Tecnológica of Pereira reviewed and approved the research as ‘research without risk’ and guaranteed the anonymity of the patients, following the Declaration of Helsinki. **References**: 1. Guariguata L, et al. Diabetes Res Clin Pract. 2014; 103:137. 2. Machado Alba JE, et al. Pan Am J Public Health. 2007; 22:125. 3. Li R, et al. Diabetes Care. 2010; 33:1872.

07.023 Fluoxetine exposure effect during pregnancy and lactation on corticotrophic axis in rats. Bacchi AD[1], Barbosa MA[1], Crespigio J[2], Mazzuco TL[2], Stabile GRV[3], Moreira EG[1] [1]UEL – Ciências Fisiológicas, [2]UEL – Clínica Médica, [3]UEL

Introduction: Fluoxetine (FLX) is a selective serotonin reuptake inhibitor (SSRI) widely used for depressive, anxiety and nociceptive disorders and it is the drug of choice for pregnant women. Since FLX crosses the placenta and is excreted in milk, maternal treatment with this antidepressant may expose the fetus and neonate to increased levels of serotonin (5-HT). Long-term behavioral abnormalities have been reported in rodents exposed to higher levels of 5-HT during neurodevelopment, suggesting that pups exposed to SSRIs during pregnancy and/or lactation present neurofunctional and endocrine changes that persist into pubescence and adulthood. Also, 5-HT has been reported to play a role in stress response since its discovery and the ability of the early environment to influence both basal and stress-induced activity of the hypothalamus–pituitary–adrenal (HPA) axis has gained attention. In order to expand the studies on the long lasting effects of fluoxetine exposure during pregnancy and lactation, this study evaluated in rats (male and female) exposed to fluoxetine during development the hypothalamus-pituitary-adrenal (HPA) axis in male and female pubertal rats (PND 35), by challenge with adrenocorticotropic hormone with subsequent plasmatic corticosterone quantification as well quantification for ACTH (in pituitary) and 3β-hydroxysteroid dehydrogenase - HSD3β, a key enzyme in corticosterone synthesis (in adrenal). **Methods**: Pregnant rats were allocated into 2 groups and received daily by gavage tap water (CON) or 5 mg/kg of FLX since the beginning of gestation until the weaning. FLX and control, male and female pups, were tested in postnatal day 35 by ACTH challenge (50 mg/kg ACTH or saline, sc) and subsequent plasmatic corticosterone quantification by enzyme immunoassay as well by quantification for ACTH (in pituitary) and HSD3β (in adrenal) by immunohistochemistry. **Results**: There was no significant reduction in plasma corticosterone levels in response to ACTH stimulation, but the HSD3β enzyme expression was reduced in animals exposed to FLX (Male Control: 0.26±0.05; Male FLX: 0.09±0.01*; Female Control: 0.14±0.03; Female FLX: 0.08±0.02*; *$p<0.05$), indicating impairment in the adrenal biosynthesis of glucocorticoids. The expression of ACTH in the pituitary, on the other hand, was not altered by exposure to FLX during development. **Conclusion**: The present study suggests that exposure to FLX can lead to changes in corticosterone biosynthesis, not followed by changes in plasma corticosterone levels or pituitary ACTH immunostaining.