07. Endocrine, Reproductive and Urinary Pharmacology

07.001 Implantation and standardization of obesity induced by high glycemic index diet in wistar rats. Ferreira SRD, Pessoa RF, Moura TMCF, Bezerra CO, Lima JPM, Cavalcante HC, Aquino JS, Cavalcante FA UFPB

Introduction: Obesity is defined as abnormal or excessive accumulation of fat and affects a large number of individuals (WHO, Obesity and overweight, 2018). It is a complex and chronic medical condition causing impact on morbidity, mortality, cost of health care (HU, F. B., Arch Intern Med, v. 167, p. 875, 2007), and is risk factor for many diseases (JAGRITI, U., Med Clin N Am, v. 102, p. 13, 2018). To understand physiopathology of obesity various animal models have been used to emulate like condition in humans and diet was one of them (KUMAR, S., Pharm Biol, v. 51, p. 607, 2013).Thus, the aim of this study was implanting and standardizing the methodology of obesity induced by high glycemic index (HGLI) diet in Wistar rats. **Methods**: To experimental protocols were used Wistar rats. These animals were randomly divided into control group (CG), which received a standard diet and obese group (OG), fed with a HGLI diet during 16 weeks. For the preparation of 100 g of this diet, 45.2 g of Labina®ration was grounded using a food processor, adding 9.6 g of refined sugar and 45.2 mL of condensed milk, followed by manual homogenization in the form of cylinders, dried in oven at 55 °C, for 24h, and offered to animals (Adapted of LUZ, A. B. S. Biosci rep, v. 38, p. 1, 2018).All experimental protocols were approved by Ethical Committee of in Animal use of UFPB (1162100918). All results were expressed as mean ± standard error of the mean (S.E.M.) and statistically analyzed using Student's t test using GraphPad Prism® software version 5.01. **Results**: The CG group presented an initial body mass of 230.0 ± 7.1 g, not differing from OG, which showed an initial body mass of 240.8 ± 13.3 g. However, after the 16 weeks there was an increase in final body mass of OG (418.5 ± 18.5 g) in relation to CG (342.9 ± 12.4 g). Interestingly, average food intake weekly was not increased in the groups (166.4 ± 3.8 g and 169.3 ± 3.9 g, respectively), so probably the highest energy in the diet was responsible for increased of body mass, even without higher food intake. The naso-anal length was also measured and OG showed increased of this parameter (28.2 ± 0.4 cm) when compared with GC (25.6 ± 0.1 cm). Although there were no differences between GC and OG in values of fasting blood glucose (100.0 ± 3.3 mg/dL and 92.2 ± 10.5 mg/dL, respectively), abdominal (20.5 ± 0.2 cm vs 20.4 ± 0.5 cm) and thoracic circumferences (17.9 ± 0.4 cm vs 19.2 ± 0.9 cm), Lee index (0.28 ± 0.00 g/cm vs 0.28 ± 0.00 g/cm) and body mass index (0.58 ± 0.01 g/cm^2 vs 0.62 ± 0.04 g/cm^2). On another hand, other parameters have been changed, included adipose tissue. OG increased retroperitoneal (15.8 ± 1.6 g) and epididimal (13.1 ± 2.2 g) adipose tissues mass when compared with GC (7.2 ± 0.5 g and 6.4 ± 0.4 g, respectively). However, inguinal adipose tissue was not altered in GO (9.2 ± 2.0 g) as can observed in comparation of CG (6.1 ± 0.6 g). Even so, there was an increase in the adiposity index, being greater in GO (7.7 ± 0.5%) than in group GC (5.1 ± 0.3%). **Conclusions**: These results allow concluding that the hyperglycemic diet-induced obesity model was successfully established, allowing other investigations about physiopathology of obesity. **Financial support:**CNPq, PPgPNSB/CCS/UFPB.

07.002 N-acetylcysteine and alpha-lipoic acid improve oxidative stress, inflammation and serum lipids levels in ovariectomized rats via estrogen-independent mechanisms. Delgobo M, Agnes JP, Gonçalves RM, Santos VW, Zanotto-Filho A UFSC

Introduction: The clinical manifestations of decreased levels of estrogen and progesterone in menopause and post-menopausal women result in a sub-chronic low-level oxidative stress, inflammation, dyslipidemia and decreased metabolism. These pro-oxidant and inflammatory conditions were demonstrated in previous studies with menopausal women as well as were validated in experiments with ovariectomized (OVX) rodents. Hormone replacement therapies are effective in many instances, even though many patients either do not respond or are not eligible. The aim of this study was to evaluate the impact of short-(15d) versus long-term (60d) sexual hormone depletion and whether antioxidant supplementation with N-acetylcysteine (NAC) and alpha-Lipoic Acid (LA) provide benefit upon oxidative stress, metabolic, and inflammatory parameters in OVX rats. **Methods:** Female *Wistar* rats (90 days), N=8/group (CEUA number: 2231170317) were divided in groups (sham-operated; OVX+vehicle, OVX+E2 (E2: 17-beta-estradiol, 20µg/Kg/day), OVX+NAC or OVX+LA at 10 to 50 mg/Kg for 15 and 60 days. Liver, kidney and heart were collected and analyzed the markers of i) oxidative stress (Thiobarbituric acid reactive substances (TBARS); protein Carbonyl and Sulfhydryl; Glutathione (GSH); Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPX); Glutathione redutase (GR) and Glutathione S-transferase (GST) activities), ii) Inflammation (Interleukin-6 (IL-6) and Tumor necrosis factor alpha (TNF-α) by ELISA), iii) lipid metabolism (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides) as well as changes in body weight; retroperitoneal fat and uterine weight were evaluated and iv) NRF2 immunoblots. **Results:** Short-term OVX rapidly depleted circulating estrogen, causing uterine atrophy and body weight gain without affecting oxidative damage, inflammatory and lipid metabolism markers, in contrast, long-term OVX augmented oxidative damage in serum and peripheral tissues as well as increased serum total cholesterol, TNF-α and IL-6 levels. The long-term OVX-induced oxidative stress was associated with depletion of GSH and total non-enzymatic antioxidants as well as decreased activity of GPxandGR. SOD and CAT activities were not altered. NAC and LA supplementation prevented GSH and non-enzymatic antioxidants depletion as well as restored GPx and GR activities, TNF-α and IL- 6 in OVX rats to levels similar to sham-operated rats. NAC and LA effects appear to be independent on NRF2 activation or estrogen-like activity, since NAC/LA did not promote NRF2 activation and were not able to emulate estrogen effects in OVX rats and estrogen receptor-positive cells. **Conclusion:** NAC and LA may improve some deleterious effects of hormone depletion in menopause in an estrogen-independent manner. These preclinical evidences are provocative for further evaluation of NAC and LA in menopause patients, especially those who experience adverse effects or are not eligible for hormonal treatment (Publication doi:10.1016/j.jnutbio.2019.02.012).

07.003 Metyrapone Treatment Reduces maternal corticosterone, however does not reverse the placental changes resulting from maternal food restriction in Wistar Rats. Gil NL[1], Severo PH[1], Azevedo GA[1], Balbino AM[1], Landgraf MA[2], Landgraf RG[1] [1]Unifesp-Diadema, [2]UNIP

Introduction: The intrauterine environment integrity has been associated a safe gestation, moreover it could prevent the occurrence of some diseases in adult life. The placenta, as an organ which establishes the maternal-fetal interface, under maternal stress condition is liable of structural and functional alterations, resulting in reduced fetal growing. Previous studies showed maternal glucocorticoids increasing due to maternal food restriction. The aim of this study is investigating the placental alterations of wistar rat dams as effect of food restriction and increased glucocorticoids during pregnancy and the implications on fetal development. **Methods:** One male for three females wistar rats were housed for one night together, and after confirmed the mating, the first day of gestation was determined and the females were divided in three groups – control dams which received ad libtum chow, restricted dams which received 50% of chow in relation to the control consumption and restricted dams which received 50 % of chow in relation to the control consumption treated with metyrapone (MET – 0,5mg/mL) v.o. On the twentieth day of pregnancy, caesarean section was performed - the dams serum were collected, and after euthanasia, the placenta and puppies were withdrawn and weighed. The placenta tissue was used to evaluate glucocorticoid receptor (GR) expression by western blot and histological analysis. **Results:** Throughout pregnancy, control dams presented greater weight gain. The corticosterone quantification in serum showed increased levels in restricted dams while restricted dams treated with MET presented levels similar to the control dams. The adrenocorticotropic hormone (ACTH) and GR expression were increased in restricted and restricted + MET dams. Both offspring of restricted and restricted + MET dams presented low birth weight, moreover, these two groups presented placentas with reduced weight. It was possible to observe in placenta histological analysis the synciciotrophoblastic barrier thickening in the labyrinth region of restricted and restricted+MET dams. **Conclusion:** The MET treatment during pregnancy inhibited corticosterone synthesis as expected, decreasing the exposure of maternal glucocorticoids on fetuses, however, the placental alterations observed in preliminary results and the consequences on offspring birth weight are related to the food restriction itself and not to maternal stress due to food restriction. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, FAPESP (2017/02042-3 and 2019/05242-9) and CNPq.

07.004 Programming of lipid metabolism in male rats after intrauterine exposition to Dexamethasone. Souza DN[1], Veronesi VB[1], Santos-Silva JC[1], Teixeira CJ[1], Hecht FB[1], Bordin S[2], Anhê GF[1] [1]FCM-Unicamp, [2]ICB-USP

Introduction: Physiological adaptations during pregnancyare pivotal to grant appropiate fetal development with long-lasting consequences. Exposure to environmental stressors during the intrauterine life may therefore program glucose intolerance, hyperinsulinemia and hyperglycemia during adulthood. Adverse intrauterine environment in rats and humans induced by maternal stress, caloric restriction and the excess of glucocorticoids are linked with low birth weight. Although the glucocorticoids are widely used in premature birth, a few studies have shown the relationship between the glucocorticoid (GC) excess and low fatty acid absorption, lower expression of lipoprotein lipase and also a reduced small intestine length. Thus, we hypothesized that the excess of GC in late gestation could impair the lipid metabolism during adult life. So, this work aims to evaluate how the gene expressions are modulated in the process of lipoprotein formation in rats with 3-month age and, also, the probable mechanism related with low triglyceridemia in male rats that were born from rats treated with dexamethasone during pregnancy. **Methods:** Nulliparous Wistar rats were treated with dexamethasone, subcutaneously, with 0.1mg/kg/day (DEX), from the 15th to the 21th day of pregnancy. After birth, the offspring were cross-fostered intragroup and, with 3 month age, the group that was born and breastfed by a rat treated with DEX will be call as DEX/DEX, and the control group CTL/CTL. Male rats were used in respirometry test, determination of gastrointestinal transit time. The trunk blood was collected to quantify the triacylglycerol and the small intestine was used to quantify the gene expressions related to lipid metabolism and, also, analyze the fatty acid oxidation. **Results**. The expression of genes related with lipid metabolism like DGAT1, DGAT2, APOB and MTTP, at jejunum, were similar in both groups. However, the expression of CD36 was higher in DEX/DEX compared to CTL/CTL, even though the fatty acid oxidation is similar in both groups. The total gastrointestinal transit is faster in DEX/DEX. The triglyceridemia is lower in DEX/DEX. Nevertheless, there were no significant differences at the respirometry test. **Conclusion**. Male offspring from rats treated with dexamethasone in late gestation showed, in adult life, a reduced capacity to absorb free fatty acid, probably due to a lower expression of CD36 in small intestine (jejunum) in DEX/DEX and, added to a faster gastrointestinal transit time, could be related to the lower triglyceridemia. **Financial Support**: FAPESP, Capes, CNPq *Approved by Ethics Committee of University of Campinas, Nº 4346-1

07.005 *In vitro* **effects of fluoxetine on rat distal cauda epididymis contraction**. Samala M, Melo AB, Mateus F, Pontes THA, Gomes LTC, Gavioli EC, Silva Júnior ED
UFRN
Introduction: Fluoxetine is a Selective Serotonin Reuptake Inhibitor widely used for the treatment of depressive disorder. Several studies have been described that fluoxetine is able to impair male fertility by decreasing the quality and the number of sperm cells in the ejaculate. However, the mechanism by which fluoxetine affect male fertility is still not fully understood. It is also reported that alterations in epididymis contraction induced by different drugs is able to affect the number and the quality of sperm cells leading to alterations in male fertility. Therefore, this study was carried out in order to investigate if fluoxetine is able to affect epididymis contraction induced by exogenous agonists or KCl. **Methods:** Distal cauda epididymis segments from adult male Wistar rats were isolated and mounted in a standard organ bath preparation. After 30 min of stabilization period, time-course response for KCl80 mMor cumulative concentration response curves for phenylephrine or carbachol were performed in the absence or presence of fluoxetine (1, 3 or 10 uM; preincubated for 30 min). The pharmacological parameters E_{max} and pEC_{50} (potency) were determined for phenylephrine or carbachol. All procedures were approved by UFRN Animal Ethics Committee (protocol number: 0058/2018). **Results:** Fluoxetine 3 and 10 uM were able to depress the contractions of distal cauda epididymis induced by KCl by about 30 and 70%, respectively. Fluoxetine 3 and 10 uM decreased the E_{max} for phenylephrine by ~50 and ~80%, respectively. We also found that fluoxetine 1uM increased in~3-fold the potency for phenylephrine while fluoxetine 10 uM significantly decreased the potency for this agonist (~5.5-fold). Similarly, fluoxetine 3 and 10 uM also diminished the E_{max} for carbachol (~50 and ~75%, respectively). Thus, fluoxetine 10 uM decreased the potency for carbachol by about 7.5-fold. **Conclusion:** Fluoxetine presented a dual effect in motor activity of distal cauda epididymis characterized by a potentiation of phenylephrine-induced distal cauda epididymis contraction at low concentration (1 uM) and a non-specific decrease in epididymis contractions induced by different agents at higher concentrations (3-10 uM). Altogether, these data could indicate that fluoxetine has direct effects epididymis contractions which could contribute to its antifertility effects reported in humans. **Financial support:** Propesq/UFRN; CAPES and CNPq. **Keywords:** Fluoxetine; distal cauda epididymis; contraction;

07.006 Food Supplementation with *Spirulina platensis* restores the damage caused by the hypercaloric diet on the relaxing cavernous reactivity of Wistar rats. Diniz AFA, Ferreira PBF, Souza ILL, Lacerda Júnior FF, Cavalcante FA, Silva BA, Silva MCC
UFPB

Introduction: Changes in body adiposity, mainly due to energy imbalance, are associated with several health problems and become risk factors for obesity and sexual dysfunctions, such as erectile dysfunction (ED) (Alves, RBCS, v.16, p. 110, 2012). Recently, it has been demonstrated that dietary supplementation with *Spirulina platensis*, a blue-green microalga, in rats fed a high calorie diet prevents the increase of reactive oxygen species (ROS), with consequent reduction of damage to erectile function (Souza, Front Physiol, v.8, p. 1, 2017).In this context, we intend to evaluate if food supplementation with *S. platensis*, restores the damage caused by the hypercaloric diet to the relaxing cavernous reactivity. **Methods:** Wistar rats (8 weeks age) were divided in rats that received standard diet (PD), hypercaloric diet (DHC) or hypercaloric diet + orally supplementation with *S. platensis* powder at25, 50 or 100 mg/kg (DHC25, DHC50 and DHC100). Animals received different diets for 16 weeks and started the supplementation with the algae on week 8, except DP that received saline solution. Cavernous reactivity was monitored. Results were expressed as mean and standard deviation of the mean and analyzed by one-way ANOVA followed by the Tukey post-test (n=5). **Results:** The relaxing efficacy of ACh was reduced in the DHC group (E_{max} = 53.5 ± 1.5%) when compared to the DP group (E_{max} = 72.7 ± 3.3%). In the DHC group, supplementation at the doses of 25 (E_{max} = 57.2 ± 5.7%) and 100 mg / kg (E_{max} = 60.2 ± 6.2%) did not alter the relaxation promoted by ACh, when compared to DHC group (E_{max} = 53.5 ± 1.5%). Interestingly, supplementation with 50 mg/kg (E_{max} = 75.9 ± 2.7%) of the algae restored the relaxing efficacy of ACh compared to the DHC group. Regarding the mechanism of action involved in the changes in relaxant reactivity, an increase in the relaxing efficiency of ACh in the DP group was observed in the presence of tempol, a SOD mimetic (E_{max} = 90.7 ± 6.9%) and that none in the presence of apocynin, an inhibitor of the NADPH oxidase complex (E_{max} = 57.6 ± 2.8%) when compared to the absence of these substances (E_{max} = 72.7 ± 3.3%). The relaxing efficacy of ACh was increased in the DHC group in the presence of tempol (E_{max} = 84.4 ± 6.6%) when compared to the DHC group in the absence of this substance (E_{max} = 53.5 ± 1.5%). In addition, no changes in efficacy and relaxing potency were observed in the presence of the apocynin of this agonist (E_{max} = 55.2 ± 4.5%, pCE50 = 8.5 ± 0.3) when compared to the absence of this inhibitor (E_{max} = 53.5 ± 1.5%, pCE50 = 7.9 ± 0.1). Food supplementation with 50 mg/kg of algae in the DHC group did not alter the relaxing efficacy of ACh, both the presence of tempol (E_{max} = 78.4 ± 5.8%) and apocynin (E_{max} = 62.3 ± 1.8%). However, there was a potentiation of the relaxing response of this agonist in the presence of tempol and apocynin (pCE_{50} = 8.4 ± 0.3 and 8.2 ± 0.2, respectively) when compared to the absence of these substances (pCE_{50} = 7.1 ± 0.2).**Conclusion:** Supplementation with Spirulina platensis restores damage to cavernous relaxing reactivity in Wistar rats fed a hypercaloric diet, by activation of the SOD enzyme and inhibition of the NADPH oxidase complex. **Financial support**: CNPq, CAPES, PPgPNSB/UFPB. Research approval: Ethical Committee on Animal Use/UFPB (6061090318).

07.007 Identification of potential EPPIN-biding proteins in murine spermatozoa. Raimundo TRF, Mariani NAP, Andrade JJ, Silva AAS, Andrade AD, Kushima HK, Santos LD, Silva EJR Unesp-Botucatu

Introduction: EPPIN (epididymal protease inhibitor), is a cysteine-rich protein containing both Kunitz and Whey-acidic protein (WAP)-type four disulfide core protease inhibitor consensus sequences. EPPIN is found on the surface of spermatozoa and is considered a promising target for male contraception due to its critical role in sperm motility. Considering the development of novel EPPIN-binding drugs, animal models are required to test their safety and efficacy in vivo in pre-clinical trials, as well as to further investigate EPPIN functions. For that, we explore the mouse as an experimental model, considering that EPPIN primary sequence is highly conserved between humans and mice, reaching ~80% identity in their C-terminal region. Moreover, the EPPIN expression profile is conserved between mouse and human. Herein, we identified the potential EPPIN interacting proteins on mouse mature spermatozoa. **Methods:** Adult (90 days-old) male C57BL/6 mice were euthanized by inhaled isoflurane overdose. For the preparation of sperm protein extract, we collected ~14.0 x 10^6 spermatozoa from corpus/cauda epididymis and incubated them with the phosphate buffer-soluble fraction of seminal vesicle fluid from the same animals. We performed co-immunoprecipitation assays using 5 µg of anti-EPPIN Q20E antibody or rabbit IgG (negative control) to spermlysate, using the MS Compatible Magnetic IP Protein A/G kit (Pierce, cat #90409).We processed the samples forprotein digestion with trypsin, and label-free mass spectrometry (LC-MS/MS)analysis using an Ultimate 3000 LC liquid nanocromatography equipment coupled to the Q-Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer. We also performed Western blot studies using affinity purified anti-EPPIN, anti-SVS2 and anti-SVS3 antibodies. **Results:** Co-immunoprecipitation with anti-EPPIN antibody following by LC-MS/MS analyses using protein extracts from spermatozoa pre-incubated with seminal vesicle fluid, revealed the presence of tryptic peptides: KSGGSAFGQVKS and KSYAAQLKS corresponding to seminal vesicle-secreted proteins SVS2 and SVS3, respectively. None of these proteins were identified in the negative control assays.The identity of SVS2 and SVS3 in co-immunoprecipitates with anti-EPPIN antibody was further confirmed by Western blot using anti-SVS2 and anti-SVS3 antibodies. **Conclusion:** Mouse EPPIN is a protein hub for seminal vesicle secreted proteins SVS2 and SVS3 on sperm surface. Considering the roles of SVS2 and SVS3 on the modulation of mouse sperm capacitation and acrosome reaction, we propose that EPPIN-SVS2 and EPPIN-SVS3 interaction plays important actions on sperm physiology, since both SVS proteins are inhibitors of sperm capacitation and acrosome reaction. **Support:** Fapesp (2017/11363-8, 2015/08227-0). **Ethics Committee approval**: 1049-CEUA.

07.008 Chronic treatment with fluoxetine or sertraline affected epididymal contraction and sperm parameters. Silva Júnior ED, Bezerra MS, Martins ABM, Trajano FMG, Pontes THA, Gomes LTC, Gavioli EC UFRN

Introduction: Antidepressant drugs, including Selective Serotonin Reuptake Inhibitors (such as fluoxetine or sertraline), are able to impair the sexual function (libido, ejaculation) and semen quality, decreasing the male fertility. However, the exact mechanism behind these effects is still not fully understood. In this study, we sought out to investigate if fluoxetine or sertraline could induce part of their anti-fertility effects by affecting epididymal contraction, and consequent sperm count and transit time trough epididymis. **Methods:** Adult Male Wistar rats were treated with fluoxetine (20 $mg.Kg^{-1}$, i.p.), sertraline (20 $mg.Kg^{-1}$, i.p.) or drug-free vehicle (control group) for 21 consecutive days. At the end of the treatment, animals from different groups were euthanized and the epididymis used for: (a) the analyses of spontaneous or exogenous agonists (phenylephrine or carbachol)-induced distal cauda epididymis contractions in an isolated organ bath set up; (b) determination of sperm parameters (sperm count and transit time in head/body or cauda epididymis). Testes were also isolated for the determination of daily sperm production and transit time trough epididymis. The results were expressed as Mean±SEM from at least 6 experiments. The contractile effects of phenylephrine or carbachol were analyzed by cumulative concentration response curves followed by determination of pharmacological parameters E_{max} and pEC_{50} (potency). All procedures were approved by UFRN Animal Ethics Committee (protocol number: 0058/2018). **Results:** The fluoxetine treatment was able to increase the frequency and amplitude of spontaneous distal cauda epididymis contractions while sertraline treatment only augmented the amplitude of these contractions. Both fluoxetine and sertraline treatment increased the potency of phenylephrine by about 3-fold without altering the E_{max}. Carbachol induced distal cauda epididymis contractions were unaffected by chronic treatment with fluoxetine or sertraline. We also found that both fluoxetine and sertraline treatments significantly decreased the testicular sperm daily production and sperm count in head/body and cauda epididymis. Moreover, sperm transit time trough epididymis cauda was accelerated after chronic treatment with fluoxetine or sertraline. **Conclusion:** Fluoxetine and sertraline are able to affect epididymal contraction and this effect could contribute to the low sperm count (plus diminished daily sperm production) and accelerated transit time trough epididymis found after chronic treatment with these drugs. **Financial support:** Propesq/UFRN; CAPES and CNPq. **Keywords:** Fluoxetine; Sertraline; Epididymal contraction; Sperm parameters.

07.009 Protective effects of LASSBio-788, a potential antiatherogenic compound, on reproductive function in hypercholesterolemic male rats. Maia IC[1], Gontijo LS[1], Moreira TJ[1], Motta NAV[1], Ribas JAS[1], Kummerle AE[2], Brito FCF[1], Marostica E[1] [1]UFF, [2]UFRRJ

Introduction: Atherosclerosis is closely associated with inflammatory and immune responses, activation of platelet aggregation and increase of oxidative stress, besides to cause deleterious effects on the reproductive tract impairing fertility (Shalaby et al., Pharmacol Res 50:137, 2004). LASSBio-788 is a thienylacylhydrazone derivative that has an antiatherogenic effect with antiplatelet, anti-inflammatory, vasodilatory, antioxidant and hypolipidemic properties well established (Motta et al., J Pharmacol Sci 123:47, 2013). Thus, the aim of this study is to evaluate the possible effects of LASSBio-788 on male reproductive tract of hypercholesterolemic rats. **Methods:** (CEUA 695/16) Male Wistar rats (60-day old) were randomized in 4 groups (n=6/group), receiving standard chow (CO) or hypercholesterolemic diet (HC) for 45 days. In the last 15 days, rats were treated with LASSBio-788 100 µmol/kg, i.p. (CO+788 or HC+788), whereas the other groups were treated with vehicle i.p. (CO or HC). After 45 days of experiment, animals were anesthetized and testes from different experimental groups were removed, weighed and processed for morphometric analyze (area, diameter and epithelial height of seminiferous tubules). Testicular cells were dissociated for assessment of apoptosis (annexin V and propidium iodide labeling) and fixed for evaluation of the DNA content of germ and somatic cells by flow cytometry. Spermatic evaluation (motility, sperm number, membrane integrity and hypo-osmotic swelling test) was performed using sperm from epididymis cauda. Values are mean±SEM (one-way ANOVA, Newman-Keuls; $P<0.05$). **Results:** LASSBio-788 increased relative weights of the testis when administrated with the HC diet. Morphometric analysis of the testes showed that LASSBio-788 partially recovered deleterious effects of HC diet on the height of the seminiferous epithelium. Furthermore, HC diet reduced the proportion of haploid cells, increasing the proportion of tetraploid cells, indicating impairment in spermatogenesis efficiency, which was fully recovered by LASSBio-788. In addition, HC diet induced apoptosis of germ cells that was avoided with LASSBio-788 treatment. Regarding spermatic evaluation, LASSBio-788 partially recovered HC diet effects on the male gamete. **Conclusion:** Our results showed that the new compound LASSBio-788 was effective in recovering deleterious effects induced by hypercholesterolemic diet in on male reproductive tract. Thus, this potential antiatherogenic compound could contribute to maintenance of fertility in hypercholesterolemic patients. **Financial Support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

07.011 The Epididymal protease inhibitor (EPPIN) sequence 111QGNNNNFQSKANC123 is critical for its role as a modulator of mouse sperm motility. Silva AAS[1], Mariani N[1], Raimundo T[1], Avellar MCW[2], Kushima H[1], Silva EJR[1]
[1]Unesp-Botucatu, [2]Unifesp-EPM

Introduction:EPPIN (Epididymal protease inhibitor) is a sperm surface protein considered a promising male contraceptive drug target due to its key role on sperm function. Upon ejaculation, the seminal plasma protein SEMG1 binds to EPPIN C-terminus on sperm surface, temporarily inhibiting sperm motility. It has been shown that both anti-EPPIN antibodies and small organic compoundstargeting EPPIN C-terminal region mimic the effects of SEMG1 and inhibithuman sperm motility. Here, we identify EPPIN's sequence involved in the modulation of mouse sperm motility by investigating the effects of two anti-EPPIN antibodies (S21C and F21C Abs) mapping EPPIN C-terminal regions on sperm motility parameters. **Methods:** Adult (90-130 days old) male C57BL/6 mice (n=3-4/group) were euthanized and their cauda epididymis were removed, placed in HTF medium supplemented with 0.75% BSA (37ºC, 5% CO2), and cut with scissors to release spermatozoa. Sperm suspensions were adjusted to 2.5 x 10^5cells/ml and wereincubated in HTF medium containing 0.4 mg/ml pre-immune serum (PIS, control) or the following anti-EPPIN Abs: S21C and F21C, raised against peptides ^{103}SMFVYGGAQGNNNNFQSKANC^{123} and ^{90}FLHWWDKKDNTASMFVYGGC^{110} of human EPPIN, respectively. The ability of S21C and F21C Absto bind mouse EPPIN was investigated using spermatozoa processed for Western blot and immunofluorescence assays. Sperm motility was assessed by computer-assisted sperm analysis at different time-points (0-120 min) after incubation. Sperm tracks were classified as motile, progressive, and static. The following sperm kinematic parameters were analyzed: average path velocity (VAP; µm/s), straight line velocity (VSL; µm/s), curvilinear velocity (VCL; µm/s), amplitude lateral head (ALH; µm), straightness (STR, %) and linearity(LIN; %). Hyperactivated motility was determined when: VCL ≥ 238.5µm/s, ALH ≥ 9.5µm and LIN < 23.6%). Data were analyzed by Student's t-test; $p<0.05$ was considered significant. **Results:** Western blot analysis revealed that both F21C and S21C Abs recognized EPPIN as a ~14 kDa band. Immunofluorescence assays showed that both Abs detected EPPIN on sperm head and flagellum. Motility of spermatozoa incubated with F21C Ab was similar to control at all time-points analyzed. S21C Ab, however, reduced sperm motility (motile and progressive) in comparison to control after 60 min (control vs S21C; %motile: 77.8± 3.5 vs 58.4 ± 2.0; %progressive: 48.9 ± 3.8vs 28.1 ± 1.9).At this time-point, S21CAb decreased sperm kinematic parameters that described vigour (VCL: 234 ± 17.9 vs 163.7 ± 19.7 and ALH: 18.9 ± 1.4 vs 13.6 ± 1.7) and progressive (VAP: 108.7 ± 6.8 vs 75.6 ± 9.6; and VSL: 68.4 ± 5.9 vs 44.6 ± 4.7) movements. Similar results were observed after 90 and 120 min of incubation. S21C, but not F21CAb,reduced hyperactivated motility in comparison to control 90 min after incubation (11.2% ± 5 vs 5.2% ± 4).**Conclusions**: Our results showed that blocking EPPIN C-terminus on mouse sperm surface results in the inhibition of sperm motility, indicating EPPIN's role in sperm function is conserved between mice and humans. Furthermore, EPPIN's C-terminal sequence ^{111}QGNNNNFQSKANC^{123} plays a crucial role in the modulation of sperm motility and hyperactivation in mice. Our study provides new insights into EPPIN function and evolution, as well as in the validation of the mouse as a suitable experimental model for translational studies on EPPIN. **Financial Support:** Fapesp (2015/08227-0 and 2017/20499-0). **Local Ethics committee approval**: 703-CEUA.

07.012 The multidrug resistance protein inhibitor, MK571 reduces prostate smooth muscle contractility from obese mice and patients with benign prostatic hyperplasia. Bertollotto GM, Oliveira MG, Passos GR, D´Ancona CA, Antunes E, Mónica FZ Unicamp

Introduction: The intracellular levels of cyclic nucleotides cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) are controlled by their rate of formation and degradation. Phosphodiesterases and efflux transporters are the main mechanisms responsible to control the intracellular levels. Our previous study in prostate, bladder and urethra from healthy mice showed that the non-selective multidrug resistance protein type 4 and 5 inhibitor, MK571 increased the relaxing responses induced by cGMP- (urethra and prostate) and cAMP- (bladder) increasing substances (Bertollotto et al., 2018). Because prostates from obese mice and from patients with benign prostatic hyperplasia (BPH) present greater smooth muscle contractility, this study is aimed to evaluate the effect of MK 571 in isolated prostate.

Methods: C57BL/6 male mice fed for 10 weeks with standard chow or high-fat diet were used. Ventral prostate from lean and obese mice were mounted in myograph chambers containing oxygenated Krebs solution. After 45 min of stabilization, MK-571 (1 µM) or vehicle (water) were added and allowed to incubate for 30 min. Then, cumulative concentration-response curves (CRCs) to phenylephrine (Phe; 0.001 - 100 µM), were obtained. In another set of experiments, fresh prostatic tissue specimens were obtained from patients with BPH submitted to transurethral prostatectomy. Samples were washed with Krebs solution, cut and mounted in myograph chambers. After 45 min of stabilization, MK-571 (20 µM) and/or tadalafil (300 nM) were added and allowed to incubate for 30 min. Then, CRCs to phenylephrine (0.001 - 100 µM) were obtained. Immunohistochemistry using anti-MRP4 was also employed. GraphPad Prism was use for all analysis, and data are presented as mean ± S.D. $P< 0.05$ was considered statistically significant. **Results:** In mice isolated prostate, phenylephrine induced concentration-dependent contractions in both lean and obese groups, however, the responses were significantly higher in obese (E_{max}, 4.5± 0.3mN, n=11, $P<0.05$) compared with lean mice (E_{max}, 2.5± 0.2mN, n=6). Pre-incubation with MK-571 (1 µM, 30 min) in obese mice largely reduced phenylephrine-induced contractions (E_{max}, 2.1 ± 0.2mN, n=6, $P<0.05$), driving the responses to the levels of lean mice. Pre-incubation with MK-571 reduced prostate-induced contraction of lean and obese, but this inhibition was greater in obese prostate (E_{max}, 3.1± 0.2mN, n=3). No significant differences for the pEC_{50} values of phenylephrine were found in any group. Furthermore, in BPH human samples, phenylephrine produced satisfactory concentration-dependent contractions (E_{max}= *123.6*±68.2% of KCl 80mM-induced contractions, pEC_{50}= 5.64± 0.37, n=5). Pre-incubation with MK-571 (20 µM, 30 min) decreased significantly the phenylephrine maximum response (E_{max}= *108.7*±44.2 % of KCl 80mM N= 4) and potency (pEC_{50}= 4.39 ± 0.22, n=4). Additionally, contractile responses to phenylephrine were significantly reduced in the presence of PDE5 inhibitor tadalafil plus MK-571 (E_{max}= *76.4*±34.5 % of KCl 80mM, n=2, $P<0.05$) compared with tadalafil alone (E_{max}=103.1±96.6 % of KCl 80mM, n=2).Finally, MRP4 proteins were immunoreactive in both smooth muscle and endothelial cells (n = 4). **Conclusion:** Our findings show that inhibition of MRPs by MK-571 restores phenylephrine-induced hypercontraction in both obese mice and human with HPB, highlighting the importance of MRP transporters in controlling the cGMP levels in prostate. **Study approvals:** CEUA 4720-1; CEP 2.734.571 **References:** Bertollotto GM, de Oliveira MG, Alexandre EC, et al. Inhibition of multidrug resistance proteins by MK 571 enhances bladder, prostate, and urethra relaxation through cAMP or cGMP accumulation. J Pharmacol Exp Ther. 2018;367:138–146.

07.013 TRPM8-activation improves the responsiveness of erectile tissue: **A cool receptor with a cool activity**. Jesus RLC[1], Araújo FA[2], Vasconcelos DFSA[1] [1]UFBA, [2]CPqGM-Fiocruz

Introduction: Erectile dysfunction is frequently encountered in patients with arterial hypertension. Due a low pharmacological response to phosphodiesterase type 5 (PDE-5) inhibitors in patients with vascular endothelial damage, the search for new drugs and therapeutic targets is of paramount importance to ED treatment.It has already been demonstrated that the activation of the TRPM8 channel was able to improve vascular function in hypertension. In previous studies conducted by our research group, we have demonstrated that TRPM8 activation induces relaxation of the pudendal artery (PA) and corpus cavernosum (CC), tissues that regulate erectile function. More interesting, TRPM8 sensitivity is increased in cavernosal vasculature from hypertensive rats. The aim of this study was to assess the influence of topical and chronic treatment with menthol-containing hydrogel (TRPM8 agonist) on the erectile dysfunction already established in spontaneously hypertensive rats (SHR), in comparison with normotensive controls (wistar). **Methods:** To evaluate the activity of the TRPM8 channel, strips of corpora cavernosa (CC) isolated from normotensive rats were used. In another set of experiments, SHR and their normotensive wistar controls, both at10 weeks, were treated topically with menthol-containing hydrogel (experimental group - carbopol 940 + menthol 0.1%) or vehicle (control group - carbopol 940) for 30 days. After treatment, CC and iliac artery (IL) were isolated to subsequent studies of contractility. All experimental protocols were approved by Committee on Ethics in Animal Use - ICS/ UFBA (130/2017). **Results:** Our results demonstrated that TRPM8 activation, by a cooling compound menthol (10^{-7}–10^{-3}M), induced relaxation of CC strips pre-contracted by phenylephrine (Phe10^{-5}M).In addition, topical treatment with menthol-containing hydrogel decreased themean arterial pressure(MAP)of SHR (mmHg= 156.0±6.2, n=6) when compared to the SHR treated with vehicle (mmHg= 191.8±10.0, n=5).Furthermore, our results demonstrated that topical treatment with menthol increased the pharmacological potency of the adrenergic (Phe) and thromboxane (U46619) agonists in CC strips isolated from menthol-treated SHR animals(pD2=5.915±0.03, n=12; 6,970±0.14, n=12, respectively) when compared to control SHR(pD2=5.796±0.03, n=9; 6.347±0.21, n=9, respectively), but, menthol treatments did not change the tissue reactivity of normotensive animals. However, no significant changes were observed on the concentration response curve of muscarinic agonist (acetylcholine) and nitric oxide donor (NPS).In another set of experiments, the pharmacological potency of Phe and NPS were increased in the IL artery rings isolated from menthol-treated SHR animals(pD2=6,485±0,04, n=8;7,791±0,06, n=8, respectively) when compared to control SHR(pD2=6,305±0,04, n=7; 7,370±0,06, n=7, respectively).In addition, the concentration-response curve of norepinephrine (NE) was shifted to the right with a consequent decrease in pharmacological potency in SHR animals treated with menthol(pD2=6.079±0.09, n=8)when compared to control SHR(pD2=6,348±0,08, n=7). **Conclusion:** In summary, our data suggest that chronic application of topical menthol gel on penile tissue was able to improve the reactivity of CC and IL artery, thus appears to have therapeutic benefits in hypertension-associated erectile dysfunction. **Financial support:** CNPq, CAPES, NIDDK Diacomp Pilot & Feasibly Program and UFBA.

07.014 Effects of growth hormone on the motility of murine macrophages during aging. Porto FL, Reis MDS, Marques ALX, Borbely KS, Mendonça BS, Menezes CA, Smaniotto S UFAL

Introduction: Age-related impairments in macrophage functions have important consequences for the health of the elderly population. Resident macrophage chemotaxis is essential for homeostatic physiological functions and in the inflammatory response, where they act in the tissue to remove pathogens and dead cells. However, the migratory activity of macrophages during aging is not completely understood. The aging process is also accompanied by a reduction in several hormones including growth hormone (GH). Previous studies by our group and others showed that this hormone can modulate macrophage activities from young individuals (Su et al, J Cell Sci 15:1733, 2013; dos Santos Reis et al, Cell Bio Int 42:615, 2018),however, the biological effects of GH stimulation on aged macrophages have not yet been elucidated. Thus, we aimed to investigate the in vitro effects of GH in the adhesion and migration of macrophage populations from aged mice. Methods: Peritoneal (PM)and bone-marrow derived macrophages (BMDM)obtained from 4 months-old (young) and 12-15 months-old (old) mice were treated in vitro with 100 ng/mL of GH for 24 hours (CEUA 67/2015 and 47/2016). After treatment, cells were analyzed for cell morphology, reactive oxygen species (ROS) production, expression of integrins, cell adhesion to extracellular matrix molecules and migration in trans well chambers. Results: We observed that BMDM from old mice had an increase on ROS production when compared to BMDM from young animals, while GH-treated PMfrom old mice has decreased ROS production. We did not notice GH effects on macrophage morphology in both populations, but the cells from old mice presented longer cytoplasmatic projections than macrophages from young mice. In the cell adhesion assays, PM from old mice have increased adhesion to laminin (LN) and fibronectin (FN), like PM obtained from young mice treated with GH. BMDM obtained from old animals have decreased adhesion to LN, but GH did not alter cell adhesion on these cells. Since the adhesion was altered, we analyzed their respective integrin receptors, VLA-6 and VLA-5, but no change was observed either in young, old or in the cells treated with GH. Lastly, we verified PM and BMDM migration activity in transwell chambers when GH was used as chemoattractant factor.The BMDM from aged mice showed decreased migration when compared to BMDM derived from young mice. Differently, PM from old mice had increased migration when compared to PM from young mice. Under GH stimulation, PM from young, old and BMDM from old mice showed higher migrating cell numbers than their respective controls. Conclusion: Our results showed that PM and BMDM have different responses regarding cell adhesion and migration during aging. Also, it was demonstrated that in vitro GH stimulus can influence macrophage chemotaxis in aging, highlighting the importance of this hormone as a target in therapies that aim to restore immune system in elderly patients. Financial support: UFAL, CNPq and FAPEAL

07.015 *In vitro* **sertraline effects on rat distal cauda epididymis contraction**. Melo AB, Samala M, Mateus F, Pontes THA, Gomes LTC, Gavioli EC, Silva Júnior ED UFRN **Introduction:** Sertraline is an antidepressant drug that is able to negative affect the male fertility by decreasing both quality and number of sperm cells in the ejaculate. Nevertheless, the exact mechanism behind the antifertility effects of sertraline is still unknown. In addition, several studies demonstrated that alterations in the motor activity of epididymis could alter the quality and the number of sperm cells, impairing the male fertility. Then, this study was conducted to check if sertraline could affect the epididymal contraction induced by exogenous agonists or KCl. **Methods:** Adult male Wistar rats were euthanized and the distal cauda epididymis were isolated and mounted in an organ bath preparation. After a stabilization period of 30 min, time-course response for KCl (80 mM for 5 min) or cumulative concentration response curves for phenylephrine (10^{-8} to 10^{-4}M) or carbachol (10^{-8} to 10^{-4}M) were made in the absence or presence of sertraline (1, 3 or 10 uM, preincubated for 30 min).The pharmacological parameters E_{max} and pEC_{50} (potency) were determined for phenylephrine or carbachol to allow comparisons between groups. All procedures were approved by UFRN Animal Ethics Committee (protocol number: 0058/2018). **Results:** The contractile effect induced by a single concentration of KCl was diminished in the presence of sertraline 3 and 10 uM by about 40 and 75%, respectively. The preincubation of sertraline 3 and 10 uM also depressed the E_{max} for phenylephrine (~35 and ~70%, respectively) or carbachol (~50 and ~80%, respectively). Sertraline 10 uM significantly decrease the potency for phenylephrine by 3-fold while the potency for carbachol remained unchanged. Sertraline 1 uM did not induce any alterations in the contractions fo distal cauda epididymis induced by KCl, phenylephrine or carbachol. **Conclusion:** *In vitro* sertraline was able to affect the contractility of distal cauda epididymis by inducing a non-specific decrease in the contractions induced by different agents. Overall, our data suggest that at high concentrations (> 3 uM) of sertraline has direct effects on epididymal smooth muscle which could be related to the negative effects of this antidepressant drug on male fertility. **Financial support:** Propesq/UFRN; CAPES and CNPq. **Keywords:** Sertraline; distal cauda epididymis; contraction.

07.016 Seminal vesicle-secreted protein 2 (**SVS2**) **reduces the motility of mouse spermatozoa** *in vitro*. Andrade JJ, Mariani NAP, Silva AAS, Andrade ADA, Raimundo TRF, Kushima HK, Silva EJRS Unesp-Botucatu

Introduction: Proteins of the REST (Rapid evolving seminal-vesicle-transcribed) family, secreted by the seminal vesicles, are the most abundant components of the semen in mammals. In men, REST proteins are known as semenogelins (SEMG1 and SEMG2). After ejaculation, SEMG1 attaches to sperm surface, leading toinhibition of motility, capacitation and acrosome reaction. In mice, REST proteins include the seminal vesicle-secreted proteins (SVS1-SVS7). Mouse SVS2 is the ortholog of human SEMG1, and also inhibits sperm capacitation and acrosome reaction. However, little is known about its role on mouse sperm motility. We hypothesized that SVS2 acts as a motility inhibitory factor of mouse spermatozoa. Here, we evaluated the effects of recombinant mouse SVS2 (mSVS2) on mouse sperm motility. We also characterized the SVS2 expression profile in the male reproductive tract of mice at different stages of postnatal life.**Methods:** To evaluate SVS2 expression profile, we used C57BL/6 male mice (*Mus musculus*;20, 40, 60 and 90 days old). Animals were euthanized by overdose of inhaled anesthetic (isoflurane), their reproductive organs (testis, epididymis, vas deferens, seminal vesicle and prostate) were collected and processed for: 1) total RNA extraction, transcriptase reverse reaction (RT) and conventional or real-time polymerase chain reaction (PCR and qPCR) for the evaluation of the mRNA levels of *Svs2* and *Rps18* (ribosomal protein 18), used as endogenous control; 2) Western blot and immunohistochemistry assays using affinity purified anti-SVS2 antibody;pre-adsorbed primary antibody with blocking peptide (30-fold molar excess)was used as negative control. To evaluate the effects of mSVS2 on mouse sperm motility *in vitro*, Swiss mice (90-120 days old; n=3/group) were euthanized, their epididymis cauda were cut and incubated in HTF medium (37°C, 5% CO2), then spermatozoa were incubated in the absence (control) or presence of 5 and 10µM mSVS2. Sperm motility was evaluated using computer-assisted sperm analysis at 20 and 60 min after incubation. Data were analyzed by ANOVA followed by Tukey test; $p<0.05$ was considered significant. **Results:** We demonstrated the abundant expression of *Svs2* transcript in the seminal vesicle from adult mice, and further detected it in the cauda epididymis and vas deferens. Western blot analysis revealed the expression of SVS2 in the seminal vesicle (tissue and fluid) as an immunoreactive band of ~38 kDa. No SVS2-immunoreactive band was observed in the cauda epididymis. Specific SVS2-positive immunostaining was found in epithelial cells and seminal fluid, but not in the interstitial space and smooth muscle cells of the seminal vesicle. qPCR assays revealed an increase in the abundance of*Svs2* transcript in the seminal vesicle of 40-, 60- and 90-day old mice in comparison to 20-day old mice. Motility of spermatozoa incubated with mSVS2 was reduced in comparison to control at20 min (control vs 5 µM/10 µM mSVS2; %motile: 73.8 ± 4.6 vs 52.9 ± 12.8/20.1 ± 16.8; %progressive 35.5 ± 1.8 vs 19.0 ± 9.0/2.5 ± 2.2) and 60 min (% motile: 74.6 ± 2.9 vs 62.2 ± 5.9/20.7 ± 14.0; %progressive 41.1 ± 6.1 vs 18.9 ± 5.5/2.6 ± 2.3). **Conclusion:** The expression of SVS2is specific to the seminal vesicle in the male reproductive tract of adult mice and is regulated by androgens. Our data indicate that SVS2 potentially plays an important role in the inhibition of mouse sperm motility upon ejaculation. **Ethics Committee approval**: 1068-CEUA.

07.017 Impact of antenatal dexamethasone treatment on cellular and molecular events during Wolffian duct morphogenesis. Sousa MED, Ribeiro CMR, Avellar MCW Unifesp-EPM

Introduction: Glucocorticoids are steroid hormones that play important role in embryonic development and tissue morphogenesis. The physiological effects of these hormones are mediated by the glucocorticoid receptor (GR), which acts as a ligand-dependent transcription factor. Insufficient glucocorticoid/GR signaling can be fatal to the fetuses primarily due to impaired lung development, while its excess from chronic maternal stress or antenatal treatment with synthetic glucocorticoids may affect fetal growth and program the fetus for life-long diseases. Recently we reported the expression and function of components of glucocorticoid receptor signaling in the developing Wolffian duct, the embryonic precursor of the epididymis that is a crucial tissue in the male reproductive tract for sperm maturation. We have also observed that antenatal treatment of dams with dexamethasone (DEX, synthetic glucocorticoid) has impact on the immunodistribution of the androgen receptor (AR) and GR along the Wolffian duct of male fetuses at embryonic age 20.5 (e20.5). In the present study we have extended our analysis to better evaluate the impact of antenatal DEX on cellular and molecular events related to Wolffian duct morphogenesis. **Methods:** Pregnant Wistar rats (90 days old) were treated daily with saline (control) or DEX(DEX-Short, 1.0 mg/kg, s.c., N=6)during the embryonic age window 17.5-e 19.5. Another group was treated with DEX during the window e13.5-e19.5 (DEX-Long, 0.1 mg/kg, s.c., N=6). All Wolffian ducts were collected from fetuses at age e20.5. Tissues were processed for RT-qPCR assays for the expression profile analysis of different genes. Immunofluorescent studies were performed with antibodies against cleaved caspase-3 (apoptosis), histone H3 phosphorylated (pH3, proliferation) and actin from smooth muscle cells (SMA). Quantification of immunolocalized cells was quantified by image analysis in fluorescence microscopy. **Results:** RT-qPCR studies revealed that DEX-Short of DEX-Long treatments presented no impact on the expression levels of Gr, Ar, Il1b, In hba and TNF transcripts (N=4, $p > 0.05$) when compared to their respective controls. However, Gilz mRNA level was reduced in Wolffian ducts from fetuses from DEX-Long group when compared to control. Immunofluorescence studies revealed no change in the number of proliferative cells or cells in apoptosis when ducts from control and DEX-treated groups were compared (N=4, $p > 0.05$). A qualitative increase in the immunodistribution of SMA-positive cells was also observed in DEX-Short group. **Conclusion:** The results contribute to a better understanding of how glucocorticoids may affect the normal development of the Wolffian duct with potential consequences to epididymal development and function. **Financial support**: CAPES, CNPq, FAPESP. **Research Ethics Committee Approval**: CEUA/UNIFESP-EPM # 1776201213.

07.018 Lipopolysaccharide reduces urethral smooth muscle contractility independently of TLR4 activation: **Implication of caspase-1 and cyclooxygenase activation**. Calmasini FB[1], Alexandre EC[1], Oliveira MG[1], Silva FH[1], Soares AG[2], Costa SKP[2], Antunes E[1] [1]Unicamp, [2]USP

Introduction: Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria and acts as an important toll-like receptor 4 (TLR4) agonist, triggering an inflammatory response through innate immune system activation. In the present study, we explored the *in vitro* effects of LPS in mice urethral smooth muscle (USM) contractility and the pathways involved on this response. **Methods:** Male C57BL6/JUnib and TLR4 KO mice were used. Concentration-response curves were performed in USM in presence of LPS (500 - 62.5 µg/mL), indomethacin (10 µM), L-NAME (100 µM) and TAK 242 (1 µM). RT-PCR assay for IL-1β, NF-k and COX-2 genes were also evaluated in presence of LPS (125 µg/mL; 2 hours) and Z-YVAD-FMK (caspase-1 inhibitor; 40 µM for 40 min). **Results:** *In vitro* LPS incubation produced a concentration-dependent USM hypocontractility to phenylephrine and vasopressin. LPS-induced USM inhibition was abrogated by prior incubation with indomethacin. Conversely, L-NAME and TAK 242 failed to reverse the LPS-induced USM hypocontractility. Similarly, TLR4 KO mice were not protected from the LPS inhibitory effect. Molecular protocols indicated up-regulation ofgene expressions for IL-1β, NF-kβ and COX-2 (25%, 24% and 32%, respectively) upon LPS incubation. The inhibition of Caspase-1 prevented the increased IL-1β, NF-kβ and COX-2 gene expressions induced by LPS. **Conclusion:** *In vitro* incubation with LPS reduced mice USM contraction independently of TLR4, through a mechanism involving caspase-1and COX-2 activation. Therefore, drugs that modulate both signaling pathways might be useful to reduce the LPS-induced urethral dysfunction under inflammatory conditions. **Financial Support:** FAPESP (2016/01178-6)

07.019 Diuretic and renal protective effect of two natural xanthones in normotensive and hypertensive rats. Souza P[1], Mariano LNB[1], Boeing T[1], Silva RCMVA[1]F, Cechinel-Filho V[1], Niero R[1], Silva LM[2], Andrade SF[1] [1]Univali, [2]UFPR

Introduction: This study aimed to evaluate the diuretic effect of two compounds 3-demethyl-2-geranyl-4-prenylbellidypholine xanthone (DGP) and 1,5,8-trihydroxy-4′,5′-dimethyl-2H-pyrano(2,3:3,2)-4-(3-methylbut-2-enyl) xanthone (TDP)in rats. **Methods:** Initially, female Wistar normotensive (NTR) and spontaneously hypertensive rats (SHR) received a single oral treatment with DGP, TDP, hydrochlorothiazide (HCTZ) or vehicle (VEH). Besides, the effects of DGP and TDP in combination with diuretics of clinical use, as well as with L-NAME (a nitric oxide synthase inhibitor), atropine (a muscarinic receptor blocker) and indomethacin (an inhibitor of the cyclooxygenase) were also explored. All the urinary parameters were assessed at the end of 8-h. Posteriorly, this effect was explored in 7 days, in which NTR and SHR were treated, once a day, with VEH, HCTZ, DGP or TDP. At the end of 7 days, the urine, blood and kidney samples were collected for biochemical analyzes. **Results:** DGP and TDP were able to stimulate 8-h diuresis of both NTR and SHR, as well as electrolytes urinary excretion, at a dose of 0.1 mg/kg.The combination with HCTZ significantly enhanced DGP and TDP induced diuresis, which was accompanied by an increase of the urinary Na^+, K^+ and Cl^- excretion. DGP plus furosemide was also able to intensify the diuresis and increase the excretion of Cl^-. Instead, the pretreatment with amiloride in combination with DGP or TDP enhanced urinary $Na^+$and decreased K^+ excretion, an effect expected by this class of diuretics. Furthermore, the diuretic effect of DGP and TDP were heightened after pretreatment with L-NAME. While atropine was able to prevent DGP-induced diuresis, the pretreatment with indomethacin precluded TDP-induced diuresis. The urinary volume of both NTR and SHR after 7 days were significantly increased with the DGP or TDP treatment. This effect was associated with increased levels of urinary electrolytes excretion. The treatments did not modify the urinary pH values, nor the parameters analyzed in plasma (Na^+, K^+, Cl^- and Ca^{2+}). In addition, the treatments did not change body weight or consumption of water and food. Concerning the renal analyzes, when compared with the VEH-treated NTR group, the activity of superoxide dismutase (SOD), glutathione S-transferase (GST) and reduced glutathione (GSH) levels in kidney homogenates of the SHR group were decreased, while that the catalase enzyme (CAT) was increased and the generation of lipid hydroperoxides (LOOH) was unaltered. Both DGP and TDP augmented the levels of GSH and GST, and reduced the CAT levels, when compared with VEH-treated only SHR. TDP, but not DGP, was able to decrease the nitrite levels in kidney samples when compared to VEH-treated SHR group, a result that corroborates the findings of the enzymatic activity of myeloperoxidase. **Conclusion:** Together, these results revealed the acute and prolonged diuretic effect of the xanthones DGP and TDP, and their renal protective effect through the improvement of antioxidative capacity in the hypertensive group. **Research support:** CNPq, CAPES, FAPESC and UNIVALI. **Authorization from CEUA/UNIVALI:** 028/17p.

07.020 Evaluation of benznidazole on epididimary sperm of mice. Mazaro-Costa R[1], Barbosa CCB[1], Nishimura ANN[1], Araújo AA[1], Carn CMC[2], Pinto LSRP[2] [1]UFG, [2]UFOP
Introduction: Benznidazole (BZN) is the only drug approved for the treatment of Chagas disease in Brazil. This disease has two forms in human, the trypomastigote flagellate form that is able to reach male reproductive system (MATIN et al., 2015). However, BZN use is known to cause several adverse effects (RIAL et al, 2017; ALDASORO et al., 2018), but there are still insufficient reports about its effects on male reproduction. Thus, the aim of this study was to evaluate the effects of BZN on epididymal sperm transit. **Methodology**: 60 adult Swiss male mice were distributed in six experimental groups (n= 10, each) exposed to treatment periods as 20 and 40 days, describe as following, all groups were treated orally: a) 100 mg/kg, animals receiving 100mg/kg/dayof BZN; b) 200mg/kg, mice receiving 200 mg/kg/day BZN; c) CTRL, animals receiving vehicle solution - methylcellulose 5%. Food and water consumptions were evaluated to verify possible toxic effects of the treatment. At the end of each period of treatment the animals were submitted to euthanasia and the epididymis were collected and weighted, after were evaluated concentration and spermatic transit. Data were analyzed by two-way ANOVA followed by Tukey's test, with significance level of $p<0.05$. The experimental protocol was approved by Ceua (Animal Ethical Commission) of the UFG (# 035/18). **Results**: No toxicity effects were observed in the water ($p=0.28$) and food consumptions ($p=0.74$). In relation to epididymis mass, the caput/corpus unity showed a reduction in the dose of 200 mg/kg by 40 days compared to CTRL and 100 mg/kg groups, and with the same dose in the 20-day period ($p=0.009$).However, cauda unity not showed differences among the groups ($p=0.09$). There was a reduction in sperm concentration in the caput/corpus unity at both doses of BZN compared with CTRL at 40-day treatment and to the same dose in the 20-day treatment ($p=0.02$). But transit time evaluation in this unity showed that the 200 mg/kg dose reduced only compared to the same dose in 20-day period ($p=0.01$).In the cauda, a reduction in sperm concentration was observed at the highest dose at 40-day treatment compared to CTRL and at the same dose at 20-day period ($p=0.002$), while transit time did not differ ($p=0.39$). **Conclusion**: The results show that despite the absence of toxic effects on water and food consumptions, BZN treatment may interferes with sperm transit time as well as sperm concentration in the mouse epididymis. Such effects may result in a decrease in sperm quality and in the natural fertility of animals. SUPPORT: This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG). **References:** Aldasoro, E. J of Antimicrob Chemother. 73, 1060, 2018. Matin, D.L. Acta Tropica. 149, 15, 2015. Rial, M.S. PlosNegl Trop Dis. 11, 1, 2017.

07.021 Atomic force microscopy and raman spectroscopy identification of biophysical and biochemical differences of trophoblast cells treated with uvaol to ameliorate Group B streptococcus deleterious effects. Tenório LPG[1], Silva ECOS[2], Marques ALX[1], Allard MJ[2], Bergeron JDB[1], Sebire G[2], Souza ST[1], Fonseca EJS[1], Borbely AU[1], Borbely KS[1] [1]UFAL, [2]Université de Montréal

Introduction: Group B Streptococcus (GBS) can cause chorioaminionitis, which isan inflammation of fetal membranes that is involved in prematurity and abortion. Antibiotics are employed to contain GBS infection, although they can also harm fetal development and do not contain GBS-derived inflammation which is also deleterious. Thereof, searching natural compounds can be useful to ameliorate this problem. One interest compound is uvaol, a pentacyclic triterpene found in olive oil with anti-inflammatory and cytoprotective properties. **Objective:** We aimed to elucidate its effects on biophysical and biochemical properties on trophoblast cells incubated with inactivated GBS *in vitro*. **Method:** HTR 8/Svneo cells were pre-treated with uvaol and incubated with inactivated GBS. Phalloidin staing was employed for F-actin polymerization analysis Atomic force microscopy (AFM), was used to evaluate topography, morphology and cellular elasticity (Young's modulus); Raman spectroscopy (RAMAN)were obtained using an algorithm in the Matlab® software and main component analysis (PCA) was applied to classify the spectral differences between cell groups (*clusters*).**Results:** The phalloidin staining and topography showed that uvaol partially prevented alterations in cytoskeleton morphology and organization caused by GBS. The Young's modulus of cells treated with uvaol and incubated with GBS increased in comparison to GBS only, indicating that uvaol increase cell stiffness. RAMAN analysis showed that there were spectral differences between groups, in control group *vs.* GBS spectral changes were observed in the regions corresponding to RNA (915 and 1240 cm -1), phosphate group (960 cm -1) and proteins and lipids (1002, 1034 and 1660 cm -1). In GBS *vs*. Uvaol + GBS, the main variations occurred in the regions corresponding to the proteins (1034, 1449 and 1660 cm -1), and RNA (915 cm -1). PCA showed that *clusters* GBS vs. Uvaol + GBS had a large samples dispersion, indicating that cells were different amid studied groups, with some overlap regions. **Conclusion:** Uvaol treatment have beneficial effects in biomechanics, biochemical composition and morphology which seems to protect cells from GBS deleterious effects. Financial Support: CNPq and FAPEAL.

07.022 Investigating the mechanism of action and function of innate immunity components in the morphogenesis of the epididymis. Nishino FA, Ferreira LGA, Ribeiro CM, Avellar MCW Unifesp-EPM

Introduction: The epididymis is an organ of the male reproductive system essential for the maturation, transport and storage of viable spermatozoa for oocyte fertilization. Its embryonic precursor, the Wolffian duct (WD), differentiates during a tubular-epithelial morphogenesis process dependent on androgens. Abnormalities of this event can result in infertility later in life. It is known that the postnatal and adult epididymis from different species presents cellular and molecular components of the innate immunity that are part of epididymal immunobiology. Among them, the β-defensin family members, which are multifunctional proteins, are abundantly expressed in the epididymal epithelium. Several epididymalβ-defensins are targets of androgen modulation, and participate in events required for sperm function. Our group identified someβ-defensins expressed in the WD, such as DEFB4 (homologue of human DEBF2), which is abundantly expressed and predominantly localized in the epithelium of this tissue. DEFB2 can exert its effects by modulating the activation of TLR4 (Toll-like receptor 4) by LPS. Our hypothesis is that DEFB2 plays a role in the WD morphogenesis regulating TLR4 cellular signaling.Aim: To characterize the mechanisms of DEFB2 and effects during epididymal morphogenesis. Methods: Rat WDs collected from male fetuses at embryonic day (e) 17.5 were cultured ex vivo in basal medium with testosterone (10 nM) and supplemented with recombinant hDEFB2 (3 nM) or E. coli LPS (25 and 100 ng/mL). Washout experiments were conducted to remove hDEFB2 after 48 h of culture. After 96 h, WDs were processed for morphology analysis using HE staining and quantification of positive cells for cell proliferation and apoptosis markers in immunofluorescence assays. Results: WDs cultured in the presence of hDEFB2 for 96 h showed reduced length and a lower degree of folding compared to the WDs cultured with testosterone only, an effect reverted when the protein was withdrawn after 48 h of culture and the duct maintained in culture up to 96 h. The morphological analysis performed on these materials did not reveal significant differences between these groups. In contrast, the ducts treated with LPS 25 ng/ml and 100 ng/ml had a higher incidence of luminal dilations than in the other groups. These changes were followed by an irregularity of epithelial cell height and absence of mesenchymal cell pattern around the epithelia. These data coincide with that observed in the gross morphology analysis of WDs in organotypic culture. Furthermore, the presence of hDEFB2 in culture medium induced an increase in the number of apoptotic cells in the duct epithelium and mesenchyme. This effect was reverted after hDEFB2 washout. Conclusion: The present data point out evidences that DEFB2 may impact epididymal morphogenesis. There are ongoing work in which we are further exploring the mechanisms of action and functions of DEFB2 during these events. Thus, the work may contribute to a better understanding of how changes in innate immune components during gestation can impact male fertility in adulthood. Financial support: CNPq, CAPES, FAPESP.