P49 – Comparative Study between Two Chilling Extender of Feline Epididymal Spermatozoa. Maria Isabel M Martins[1*], Paula A P Savi[1], AlessandraT Jesus[1], Tathiana F Motheo[2]. [1]Department of Veterinary Clinics, UEL, Londrina, Brazil. [2]Department of Animal Reproduction, Unesp, Jaboticabal, Brazil.

The purpose of this study was to compare the viability of the spermatozoa recovered from the epididymis cauda diluted in two extenders, TRIS modified (E1) and Botucrio ® without cryoprotectant (E2) (Biotech Ltda, Botucatu, Brazil), maintained in container semen transport (Botu-tainer ®) for 24 hours and refrigerator at 4°C for 72 hours. Five adult cats were orchiectomized. The recovery of the spermatozoa was accomplished by compressing the epididymis cauda and part of the deferent duct on a Petri dish containing 200µl of ringer. The sample was divided into two aliquots, centrifuged (800g/10'), and the pellets were diluted in E1 or E2; thereafter each sample was divided in four parts and packed in a transportation system for 24 hours. After these time aliquots were transferred to a container with 400mL of water and kept in the refrigerator (4°C) for 72 hours. The sperm samples were evaluated for motility, vigor, sperm concentration, percentage of viable cells and morphology each 24 hours (0, 24, 48, 72, 96). The data were evaluated by analysis of variance for repeated averages, test Tuckey with $p<0.05$. The average motility (%), alive (%) were 87 ± 5.2, 73 ± 17.2 a fresh; 67 ± 15.9, 41 ± 24.3 (M1) versus 72.4 ± 9.9, 33.4 ± 16.5 (M2) at 24 hours and 38 ± 22.4, 12 ± 10.4 (M1) versus 33.4 ± 16.6, 14 ± 12.9 (M2) at 96 hours. There were no significant differences between the extenders ($p > 0.05$) at each period. In the condition of this study both extender were able to promote protection to the membrane during cooling epididymal spermatozoa. Financial Support: PIBIC UEL/CNPq. *Correspondence: imartins@uel.br

August, 18 (Tuesday) – 17:30h – 19:15h

P50 – Selection of Endogenous Control Genes for Real Time PCR (qPCR) in Three Experimental Models of Ventral Prostate (VP) from *Wistar* Rats. Fabiana Kühne, Hernandes F Carvalho*. *Department of Anatomy, Cell Biology, Physiology and Biophysics, State University of Campinas (UNICAMP), Campinas, SP, Brazil.*

Due to the complexity of the system, with different cellular types and different responses to androgen stimulation/deprivation, prostate has variations in expression of housekeeping genes. The expression of nine housekeeping genes (B-actin, PGK-1, GusB, TBP, TBP7, PPiA, GAPDH, B2m and HPRT) was quantified by qPCR (ΔΔcT method) in three experimental models of VP from *Wistar* rats: (I) androgenic deprivation by castration at 90 days after birth and analyses at 7, 14 and 21 days later; (II) 17B-estradiol administration at days 1, 3 and 5 after birth (15 mG/kG animal/day) and analyses of eVP (estrogenizated VP) at day 90; (III) epithelial cells from eVP separated by Percoll gradient. TaqMan singleplex two step reactions were performed with five male animals/group. We designed primers and probe for 18S rRNA gene, and purchased the others. Total RNA was extracted (RNAspin Mini Kit), transcribed (SuperscriptIII and oligo dT) and 20ng used in qPCR. The results were analysed: (i) measuring standard deviation among cTs, (ii) in Add-Inn Normfinder and (iii) in software Genorm. The most stable gene were PGK-1 (models I and III) and TBP7 (model II), so the most suitable to endogenous control in those experimental models. The other genes, that once were suitable as endogenous control for different experimental models, presented in our models strong variation in expression comparing controls and treatments. The importance in determining the profile expression of housekeeping genes for each biological system and experimental model before choosing an endogenous control was clearly demonstrated in this study. *Correspondence: hern@unicamp.br

P51 – Assessment of Ovarian Follicular Growth by Ultrasound Biomicroscopy (UBM) in Mice. Paulo H A Campos Jr[1,2*], Cristina A Silva[3], João G V Grázia[4], Vitor V Maffili[3], Ricardo R dos Santos[3], Bruno C Carvalho[1], João H M Viana[1,2]. *[1]Laboratory of Animal Reproduction Embrapa Dairy Cattle, Brazil.[2] CES/JF, Brazil.[3]Fiocruz-Ba, Brazil.[4]UFJF, Brazil.*

The use of imaging technologies such as ultrasound resulted in great progress in the understanding of the process of follicular growth, recruitment, dominance and ovulation in large animals. Because of the need for high-resolution equipment, however, such physiological mechanisms are less known in small animals, and information available is based only on studies using invasive techniques. The development of ultra-high frequency ultrasound devices (30-55 MHz) allows *in vivo* visualization of organs and systems in small animals such as mice and is a potential tool for physiology studies in this specie. This study aimed to assess the ovarian follicle growth in mice treated with eCG and hCG using UBM. The ovaries of C57BL/6 strand mice (n=8) were identified with UBM (Vevo 660, Visual Sonics, Toronto, ON, Canada) coupled with Real-time Micro-visualization probe (RMV 708), and follicular population was quantified and measured every 2 hours, starting 4,5 hours after hCG injection. Follicles greater than 200 µm were clearly visualized. As expected, follicle population decreased ($P<0.05$) along time ($y = -4.06x + 41.4$; $R^2=0.87$) due to ovulations. Follicles achieved a mean maximum size of 596.7±106.0 µm, 5.8±2.3h after hCG application. In 6/8 of the animals and 7/16 ovaries, however, large follicles were observed in 2 distinct moments, 5.0±1.2h and 10.4±1.0h after hCG, respectively, suggesting that mice may have ovulation waves. In conclusion, UBM can be used to study follicular dynamics and ovulation in mice. *Correspondence: phacj@ig.com.br

P52 – Carnitine Reduces Testicular Damage In Rats Treated With Doxorubicin in The Prepubertal Phase. Rodrigo R Provenza, Vanessa Vendramini, Sandra M Miraglia*. *Discipline of Developmental Biology, Department of Morphology and Genetics, UNIFESP, SP, Brazil*

Doxorubicin is a widely used antineoplastic drug included in childhood cancer therapies, which produces serious side effects including infertility. Carnitine, a trimethilated aminoacid, which naturally occurs on the epididymis, has shown cytoprotection efficacy against doxorubicin cadiotoxicity, when exogenously administered. In this study, we have therefore investigated whether carnitine protects rat seminiferous epithelium against damage caused by doxorubicin. For this target, 24 male Wistar prepubertal rats were distributed into four different groups: Doxorubicin (D – 5 mg/kg), Carnitine (CA – 250 mg/kg), Carnitine/Doxorubicin (CAD; carnitine one hour before doxorubicin injection) and "Sham" Control (SC – Saline Solution). All treatments were given by intraperitoneal route when the rats were 30 days old. The rats were submitted to euthanasia 34 days later, on the pubertal phase. Testes were immersion-fixed in Bouin's liquid and Paraplast Plus-embedded; 3µm-thick cross sections were stained with Hematoxylin and submitted to PAS + H histochemical method. Histomorphometric and stereological testicular analyses were carried out. Sperm concentration was also accessed from the fluid of the epididymis cauda. Doxorubicin-treated rats showed a significant reduction in almost all the studied parameters when compared to SC control group; reduction of sperm concentration in the epididymary fluid was also observed. Carnitine conferred some cytoprotection on the seminiferous epithelium, against doxorubicin deleterious effect. Histopathological analyses and stereological data confirm this idea since carnitine/doxorubicin-treated rats showed a higher volume density of seminiferous epithelium than those solely Doxorubicin-treated. Thus, carnitine can be a promising substance to protect the seminiferous epithelium against doxorubicin-induced damage. Financial Support: CAPES. *Correspondence: miraglia.sm@gmail.com

II Workshop on Male Reproductive Biology

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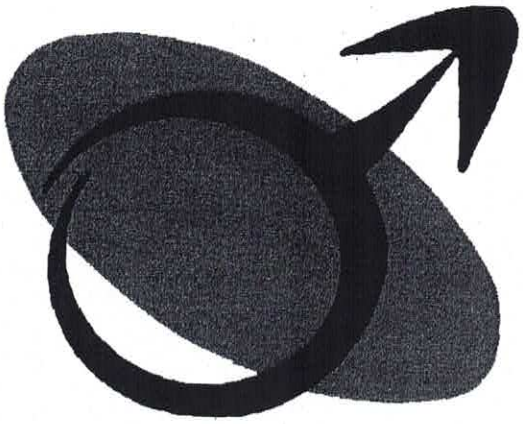

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