Reproductive Toxicology and Prostate: Role of Plasticizers, Pesticides and Toxic Metals in the Gland Histopathology Wellerson R Scarano*. Federal University of Alfenas, Department of Biomedical Sciences, Alfenas, MG, Brazil.

It has been hypothesized that chemicals with estrogenic or antiandrogenic activity may cause developmental, reproductive, and tumorigenic effects in humans and animals. Recently, a great deal of attention and interest has been directed toward the hypothesis that exposure, particularly "in utero" exposure, to certain environmental chemicals might be capable of causing adverse effects as result of endocrine modulation. Although many studies have reported the influence of toxic agents on the male reproductive system, only a few have discussed their possible effects on prostate development. It has been suggested that approximately 20% of all human cancers in adults result from chronic inflammatory states triggered by infectious agents or other environmental exposures, or by a combination of such exposures. Ours studies were based in the perinatal and adulthood exposition and its effects on the functional and histopathological aspects of the gland. We have observed that the phthalates (plasticizer), methyl-mercury, and dyuron (pesticide) exposition cause similar responses, increasing epithelial proliferation associated with inflammatory processes. In phthalate treated rats during gestation and lactation, we observed chronic inflammatory processes and epithelial reactive inflammatory atypia. Metalloproteinase-9 activity and androgen receptor expression were increased in these animals. Preliminary data showed that the methyl-mercury exposition in adult rats seems to be related to the dose: low doses induce proliferation and inflammation while high doses induce atrophy. Now, we are investigating the intracellular proliferative mechanisms AR-dependent and -independent and inflammatory cell types and molecules involved in stromal chronic prostatitis in response to environmental toxicological agents. *Correspondence: wrscarano@unifal-mg.edu.br

Poster Sessions – Abstracts

August, 16 (Sunday) – 17:30h – 19:15h

P1 – Isolation of RNA from Bovine Sperm. Alethéia C B L Souza[1], Carlos E A Souza[1], Arlindo A Moura[1]*, Gary J Killian[2]. [1]*Departamento de Zootecnia, UFC, Fortaleza, CE, Brazil.*[2]*Almquist Research Center, The Pennsylvania State University, PA, USA.*

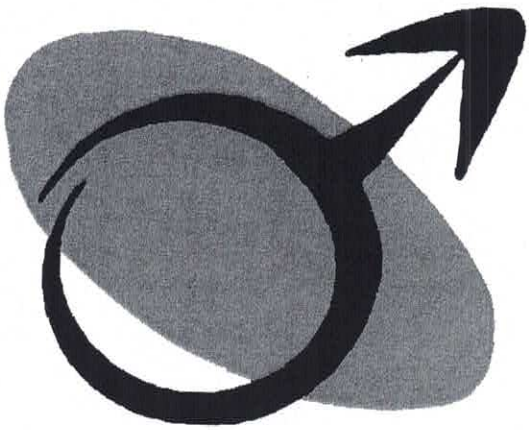
This research describes a successful method for isolation of sperm RNA from bull sperm. Semen samples from 5 mature Holstein sires were obtained and assessed for sperm concentration and motility. The samples were centrifuged (1000 *g*, 15 min., 4°C) to remove seminal plasma and the sperm pellet was resuspended in PBS and washed twice. We resuspended sperm cells in a somatic cell lysis buffer, incubated on ice for 45 min, centrifuged 3 x to remove cellular debris and rewashed them with PBS. RNA was isolated using the RNAsy kit (Qiagen Life Sciences, USA) with adaptations. Briefly, an aliquot containing 25 x 10^6 sperm was incubated in lysis buffer for 5 minutes and extensively homogenized using a 26G needle. The sample was heated (65°C) for 30 minutes, re-homogenized to break DNA and centrifuged (13.200 rpm, 3 min.) and the precipitate was discarded. Ethanol 70% (700µl) was added to the supernatant, which was loaded in the columns provided with the kit. The fraction bound to the matrix was incubated with DNAse. The column was washed again and the bound fraction was eluted with 100 µl of RNAse free water (2 x, 10.000 rpm, 1 min.). RNAse inhibitors were added to the RNA-rich sample. Quantity and quality of RNA was estimated in a spectrofotometer based on readings at 260 and 280 nm. As a result, we were able to isolate significant amounts of bovine sperm total RNA (520 to 940 µg/mL) and subject it to RT-PCR. Financial support: CAPES and USDA. *Correspondence: arlindo.moura@gmail.com

P2 – Comparison of Three Differents Methods of Sperm Separation: Swim Up, Isolate and Percoll. Juliana Polisseni[1,2]*, Bruno C Carvalho[1], Paulo H A Campos Jr, Michele M Pereira[1], Raquel V Serapião[1], Ribrio I T P Batista[1], Luiz S A Camargo[1], João H M Viana[1], Wanderlei F Sá[1], Lilian T Iguma[1]. [1]*Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil.*[2]*Federal University of Juiz de Fora , MG, Brazil.*

The separation of human spermatozoa from seminal plasma is necessary to use in assisted reproductive technologies. The aim of this work was to evaluate three methods of sperm preparation, two methods based in silica colloidal Percoll® and Isolate® and another method of sperm migration, swim up, using bovine model. It was evaluated the progressive motility and sperm vigor before and after sperm processing, viability after processing and in vitro embryo production (cleavage rate, 2, 4 and 8 cell and embryo yield). The overall sperm motility and viability and the number of embryos at the inicial stages of development were analysed with Kruskal-Wallis test. The rates of cleavage and blastocyst were compared with chi-square test. The results of sperm parameters before and after processing were similar ($P > 0.05$) in swim up, Percoll® and Isolate® (41 ± 16.73/64 ± 8.94; 46 ± 9.62/74 ± 5.48 and 48 ± 13.51/71 ± 8.94, respectively). The sperm viability after processing was similar ($P > 0.05$) between swim up (62.06 ± 21.32), Percoll® (53.09 ± 9.33) and Isolate® (64.88 ± 12.18). The percentage of 8 cells embryos (on day 3 after fertilization) was similar for swim up (37.95 ± 6.77) and Isolate® (29.71±14.10). In contrast, both methods showed higher 8-cells rate than Percoll® (18.18 ± 10.21) but Isolate® (18.18 ± 10.21) was similar ($P > 0.05$) to the mentioned treatments. The blastocyst production rate (Day 8) was higher ($P < 0.01$) when swim up was used (swim up - 48.41%; Percoll® - 25.70; Isolate® - 22.35. We concluded that swim up technique had a better performance selecting sperm and enhanced capacity for embryo production when compared with the Percoll® and Isolate® procedures. *Correspondence: jupol@powermail.com.br

P3 – Recombinant Expression of Buck (*Capra hircus*) Spermadhesin (rBdh-2) in *Escherichia coli*. Érica S Albuquerque[1], João B Cajazeiras[1], Luciana M Melo[1], Gandhi Rádis-Baptista[2], Vicente J F Freitas[1]*. [1]*Laboratório de Fisiologia e Controle da Reprodução - LFCR, Universidade Estadual do Ceará, CE, Brasil.*[2]*Instituto de Ciências do Mar - Labomar, Universidade Federal do Ceará, Fortaleza, CE, Brasil.*

The spermadhesins are male secretory proteins found in the seminal plasma able to influence diverse reproductive functions. The low purification efficiency and the incomplete characterization of buck spermadhesins (Bdhs) prompted us to establish an effective system to produce recombinant Bdhs (rBdhs). The cDNA encoding Bdh-2 spermadhesin was inserted in a prokaryotic expression plasmid pTrcHis TOPO. The pTrcHis-Bdh-2 system was constructed to produce a His_6 fusion protein in *E. coli* Top10 cells. The recombinant clones were selected by growth in ampicillin-containing medium, PCR amplifications and nucleotide sequencing. The expression of the insoluble rBdh-2 was achieved at 0.1 to 2.0 mM IPTG and after 2 to 6 h of induction. The recombinant protein synthesis was monitored by SDS-PAGE under reducing conditions followed by immunoblotting using monoclonal anti-His antibody. The image of each immunoblot membrane was assessed by densitometry using an image analysis program (ImageQuant TL 2005, Amersham Biosciences). The apparent molecular weight of rBdh-2 was 15.85 ± 0.09 kDa. This result agrees with the theoretical molecular weight of 15.5 kDa predicted from the nucleotide sequence. A significantly greater production of rBdh-2 ($P < 0.01$) occurred in the presence of 1.5 mM IPTG after 2 h of induction, and with 0.3 mM IPTG after 4h of culture. Among the induction times investigated, 6h showed the lowest levels of rBdh-2 production, where no difference was seen between the various concentrations of IPTG tested ($P > 0.01$). Thus, an effective prokaryotic rBdh-2 expression system was established to provide a good tool for studying the buck reproductive mechanisms and further applications in reproductive biotechnologies. Financial Suport: CNPq (Brasília, Brazil), FUNCAP (Fortaleza, Brazil), CAPES/PNPD (Brasília, Brazil) and International Foundation for Science (Stockholm, Sweden).*Correspondence: vjff@pq.cnpq.br



II Workshop on Male Reproductive Biology

August 16-19, 2009
Hotel Travel Inn Live & Lodge Ibirapuera
São Paulo, SP, Brazil

*** Pre-Meeting of the XXIV FESBE Annual Congress – 2009**

Official Program