BIOCELL 38 (Suppl. 2), 2014
ISSN 0327-9545 (printed version)
ISSN 1667-5746 (online version)

XV ANNUAL MEETING OF THE ARGENTINE BIOLOGY SOCIETY

(XV JORNADAS ANUALES DE LA SOCIEDAD ARGENTINA DE BIOLOGÍA)

60 years after the description of the DNA structure

December 4-6, 2013

Chascomús, Argentina

The abstracts were evaluated by the Scientific Committee of the Argentine Biology Society

BIOCELL 38 (Suppl. 2), 2014
ISSN 0327-9545 (printed version)
ISSN 1667-5746 (online version)

ARGENTINE BIOLOGY SOCIETY

Board of Directors (2013)

A71
ALLATOTROPIN AND ALLATOSTATIN-C: TWO MYOREGULATORY PEPTIDES ACTING IN THE AORTA AND CROP OF KISSING-BUGS

Villalobos Sambucaro MJ, Riccillo FL, Diambra LA, Noriega FG, Ronderos JR
Lab Cát. de Histología y Embriología Animal, Facultad de Ciencias Naturales y Museo– Universidad Nacional de La Plata (FCNyM-UNLP). E-mail: villalobos.mj2101@gmail.com

In the present study we present a partial characterization of the Allatotropin (AT) and Allatostatin-C (AST-C) receptors, also showing their miorregulatory activity in the anterior midgut (crop) and aorta in males of the kissing-bug Rhodnius prolixus. Ovary, Malpighian tubules, aorta, midgut and rectum cDNA were prepared from a pool of fed and unfed individuals. Using specific primers base on the corresponding sequences found inVectorbase, fragments of 900 and 600 bp of RpATr and RpAST-CR were sequenced. Their expression was found in every organ analyzed. For physiology assays we analized the heart rate in starved males, who were sequentially inyected with Serotonin10-9M, AT 10-9M to induce a stimulation of the frequency of contractions and AST-C 10-6 M. To further analyze the activity of these peptides, we essayed the effect of different doses of AST-C (from 10-14M to 10-6M) during post-prandial diuresis. Differences between treatments were analyzed by Multifactorial Analisys of Variance. In the group of starving insects we found that AT have a mioestimulatory funtion, causing increased heart rate, which decreased more than 20%, after applying AST-C 10-6 M. Dose-response assay in fed insects, shows that the contractions of the aorta and the crop decreased significantly during post-prandial diuresis (approximately 50% and 20%, respectively). Finally, our results suggest that the peptide AST-C would inhibit the muscle contractions of both organs,probably antagonizing the stimulatory action of AT.

A72
REGULATION OF cAMP IN *Giardia lamblia*

Zurita AR,
IMIBIO-SL(CONICET)/Area Biología Molecular, Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis. E-mail: azurita1974@gmail.com

Giardia lamblia is an anaerobic protozoan parasite that inhabits in the small intestine of humans and other higher animals, that ingested water or food contaminated with *Giardia's* cyst. It has been described that the beginning of the infection (excystation) would be mediated by the second messenger cAMP. However, the mechanisms of the cAMP regulation in *G. lamblia* has not been described. In order to enhance our knowledge of the molecular mechanisms that regulate the infection process of this parasite, we tried to to analyze how *G. lamblia* is able to regulate its own cAMP levels. As first step, during an bioinformatic analysis of the complete genome of *G. lamblia*, we identified two sequences that encode for enzymes that regulate cAMP: Adenylyl Cyclase (gAC) and phosphodiesterase (gPDE). The expression of these genes in *G. lamblia* was later confirmed by an RT-PCR study performed from trophozoites cultured in vitro. With the purpose of characterizing these putative enzymes, the sequences encoding gPDE and gAC were amplified by PCR and then inserted in expression vectors.
At the moment, we expressed and purified from bacteria three constructs of gAC truncated variants (gAC118, gAC268 and gAC301) containing its catalytic domain. The capability to syntheses cAMP of these constructs of gAC was subsequently confirmed by enzymatic assay in vitro. These results suggest that the regulatory enzymes of cAMP, gPDE and gAC are present in *G. lamblia* and that gAC would be directly involved in the synthesis of cAMP in this parasite.

ANIMAL BIOTECHNOLOGY

A73
CHOLESTEROL INCORPORATION INTO BOVINE OOCYTES PRIOR TO VITRIFICATION FAVORS RECOVERY OF GM1 LEVEL AT THE PLASMA MEMBRANE

Buschiazzo J[1,2], Ríos GL[1], Canizo JR[1], Antollini SS[2], Alberio RH[1]
[1]Biotecnología de la Reproducción, Dpto de Prod Animal, INTA-Balcarce; [2]Inst de Investigaciones Bioquímicas de Bahía Blanca. E-mail: buschiazzo.jorgelina@inta.gob.ar

Methyl-β-cyclodextrin (MβCD) saturated with cholesterol or alone was used to modulate plasma membrane cholesterol level of metaphase II bovine oocytes. Membrane biophysical state of oocytes and cumulus cells was evaluated at decreasing temperatures and changes in the localization of the raft marker GM1 were analyzed in Cryotop vitrified oocytes. After 2 hours of incubation with 15 mM MβCD saturated with cholesterol, fluorescence intensity of BODIPY-cholesterol at the plasma membrane increased. Oocytes that were incubated with MβCD alone showed no differences in membrane

fluorescence intensity but evidenced changes in the distribution of cytoplasmic lipid droplets. Laurdan generalized polarization revealed that membrane fluidity of oocytes is lower than that of cumulus cells at temperatures closer to the physiological but this relation is inverted when temperature decreases below 20°C. GM1 loss caused by vitrification was restored when cholesterol was incorporated into oocytes before vitrification and removed after warming, thus indicating that membrane raft integrity can be preserved by cholesterol modulation.

A74
THE EFFECT OF DIMETHYLUREA ON *IN VITRO* MATURATION OF PORCINE OOCYTES

Lorenzo MS, Tello MF, Cruzans P, Maruri A, Lombardo DM
Cátedra de Histología y Embriología, INITRA, FCV, UBA. E-mail: soledadlorenzo@fvet.uba.ar

A balanced redox environment is important for oocyte quality. Oocyte viability is affected by the increase of reactive oxygen species. Dimethylurea (DMTU) is a chemical antioxidant that captures free radicals derived from oxygen. The effect on *in vitro* maturation (IVM) of the addition of DMTU during follicular aspiration was evaluated. Porcine COCs were obtained by follicular aspiration from slaughter ovaries. In control, TCM 199 was used for aspiration and supplemented TCM for washing, whereas in treatments 2µM and 20µM of DMTU was added in both media. After IVM, denuded oocytes were stained with Hoechst 33342 to evaluate the percentage of nuclear maturation. Those oocytes who reached Metaphase II were considered mature. It was used an n=97 (control), n=123 (treat. 2µM) and n=137 (treat. 20µM); three replicates. Comparison of proportions (Infostat) showed no statistically significant differences. In future, the citoplasmatic maturation and the apoptosis rates will be evaluated in order to determine if the addition of DMTU as an antioxidant is convenient.

A75
ESTABLISHMENT OF A REVERSE GENETIC SYSTEM FOR THE STUDY OF AN INFLUENZA A VIRUS FROM RED-WINGED TINAMOU

Paredes Rojas Y, Caldevilla C, Matiello R, Mattion N, Ibañez LI.
ICT-César Milstein, Conicet. E-mail: iibanezcevan@centromilstein.org.ar

In 2008 an H1N1 influenza virus (A/red-winged tinamou /Argentina/MP1/2008) was isolated in Buenos Aires but so far it has not been studied at the molecular level. The goal of this work was to obtain the 8 segments of the virus by reverse genetics, with the aim of generating infectious viral particles to allow its molecular and biological characterization. After reverse transcription, the different fragments of the strain were cloned by a first PCR reaction using primers that recognize conserved sites within the non coding sequence. These fragments were used as megaprimers in a second PCR reaction using as target the pHW2000 vector, which was then treated with DpnI and transformed into DH5alpha bacteria. DNA was extracted from positive colonies and analyzed using the restriction enzyme BglI. Positive clones were already obtained for the HA fragment, while putative ones corresponding to fragments NS, M, NP and NA colonies are currently being analyzed. We have thus established a reverse genetics system to study this novel influenza virus.

A76
ARTIFICIAL INSEMINATION IN PIGS: CERVICAL VS DEEP INTRAUTERINE. INCREASING EFFICIENCY OF THE BOAR

Renoulin E G, Luchetti C G, Salamone D F.
Laboratorio de Biotecnología Animal- Facultad de Agronomía-Universidad de Buenos Aires. E-mail: caroluchetti@yahoo.com

Deep intrauterine artificial insemination (AI) is a method that optimizes boar performance with respect to the cervical AI given that it maximizes the number of possible inseminations with equal volume of semen. This technique is not still used in production and is not recommended in gilts. However, to date there are no reports of trials of this technique in gilts. In this study we compared two techniques of AI in sows: 1) cervical and 2) deep intrauterine. Both techniques were applied with cooled semen to 8 sows of similar characteristics grouped into two categories: multiparous (7 repeats, 2 inseminations per heat) and nulliparous (8 repeats, 2 inseminations per heat), to form the experimental groups some sows were inseminated more than once. There were a total of 7 cervical AI and 8 deep intrauterine AI (these last with a half of the semen volume). We compared the pregnancy rates obtained after insemination. Data were analyzed with the Fisher exact test ($p < 0,05$). No significant differences in pregnancy rates were observed, or between methods or between categories (75% in gilts with both methods and 100% in sows with both methods). The depth of semen deposition in nulliparous is lower than in multiparous.