JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences
Nutrition Physiology 2013 : 2 (Special issue) 14-17

MOLECULAR PHYSIOLOGY

PHYSIOLOGY AND GENETIC ASPECTS OF THE REGULATION OF EXPRESSION MILK PROTEIN GENES

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For the genetic improvement of milk composition and milk yield, both the typing of different protein variants and knowledge about the regulation of expression of the different milk protein genes are important. Some of the processing properties of milk are dependent on the milk composition. Information about the DNA sequence and genes involved in the expression of the milk protein genes, therefore, is big importance for genetic improvement of these traits in animals breeding programmes.In recent years more data has become available concerning the regulation of expression of the milk protein genes and as might have been expected from the complex multihormonal control of these genes it appears to be rather complex. Although several mammary gland specific factors that play a role in expression of some of these genes have been identified, none of these factors has been shown to be involved in the expression of all or the majority of the milk protein genes.

Keywords: milk, mammary gland, milk protein, genes, expression

THE EFFECT OF AMYGDALIN ON PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

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Amygdalin is a natural plant compound occurring in the seeds of apricot, almond, apples, peaches, and other rosaceous plants. Its anticancer, anti-inflamatory activity and other medicinal benefits have been known for many years. Amygdalin is composed of two molecules of glucose, one of benzaldehyde, which induces an analgesic action, and one of hydrocyanic acid, which is an anti-neoplastic compound. The aim of this *in vitro* study was to investigate the secretion activity (steroid hormone-progesterone) of health porcine ovarian granulosa cells (GC) after amygdalin administration. Granulosa cells from non-cycling and cycling porcine ovaries were incubated with amygdalin at the selected doses 1, 10, 100, 1000 and 10 000 μg/mL for 24 hours and compared to the control without amygdalin administration. The release of progesterone by GC from non-cycling and cycling porcine ovaries were assessed by ELISA. The results from our investigation showed that amygdalin did not significantly ($P \geq 0.05$) affect on the release of progesterone by porcine granulosa cells from health non-cycling and cycling ovaries at our selected doses. In conclusion, the present results suggest that amygdalin do not cause changes in secretory activity (steroid hormone-progesterone) of granulosa cells in health porcine ovaries. These findings indicate our further research of amygdalin and its effects on animal reproductive cells.

Keywords: amygdalin, porcine ovarian granulosa cells, progesterone

THE EFFECT OF GREEN TEA EXTRACT - EPIGALLOCATECHIN GALLATE (EGCG) ON BASIC OVARIAN FUNCTIONS IN PORCINE OVARIAN GRANULOSA CELLS

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Epigallocatechin gallate (EGCG) is the major catechin found in green tea (*Camellia sinensis*), but there is only little data of its influence on reproductive processes. The aim of this *„in vitro"*study was to examine the effects of green tea extract - epigallocatechin gallate (GT-EGCG) on proliferation, apoptosis and steroidogenesis of ovarian cells. Granulosa cells isolated from porcine ovaries were cultured without (control) or with GT-EGCG (0, 1, 10, 100 µg/ml of medium). Markers of proliferation (PCNA) and apoptosis (bax) were detected by immunocytochemistry. Secretion of steroid hormones (progesterone and testosterone) was measured by RIA. It was found, that GT-EGCG supplement significantly diminished percentage of cells containing PCNA (proliferation) at all used doses. Proportion of bax-positive cells (apoptosis) was increased after addition of GT-EGCG at the highest dose. Addition of GT-EGCG increased progesterone release by addition at 10 µg/ml dose and inhibited testosterone secretion at the lowest dose. In conclussion, we demonstrated that GT – EGCG has potent dose-dependent effect on proliferation and apoptosis of ovarian granulosa cells, as well as secretory activity of these cells to produce steroid hormones. It was shown, that green tee GT-EGCG can be an inhibitor of porcine ovarian functions. These direct effect on the ovary could be taken into account by green tee consumption and its application for control of animal and human reproductive procrsses.

Keywords:green tea - epigallocatechin gallate, progesterone, testosterone, proliferation, apoptosis, porcine granulosa cells

Acknowledgments: We would like to thank Mrs. Katarína Tóthová and Ing. Žofia Kuklová (Animal Production Research Centre in Nitra – Lužianky), to Mrs. Iris Stelter (Institute of Animal Science, Neustadt, Germany), to Mr. Yani Deng (Changsha Sunfull Bio-tech. Co, Hunan China) for kind of providing of green tea extract - epigallocatechin gallate . This work was financially supported by the Ministry of Education of the Slovak Republic project no. 1/0022/13, APVV no. 0137-10 and 0854-11 and no. 740531-OPVaV-2011/2.2/07-SORO.

EFFECT OF DIFFERENT CONCENTRATION OF TREHALOSE ON DOG SPERMATOZOA MORPHOLOGICAL CHANGES

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The aim of our work was to evaluate the large dog breeds ejaculate diluted with addition of trehalose with a focus on morphological changes. We used following breeds: German boxer, Greyhound, Leonberger, Border Collie. In our experiment different concentration of trehalose and cultivation temperature were used: 20 mg/ml – TA; 5 mg/ml – TB; 1.25 mg/ml – TC; 0.312 mg/ml – TD; control group – TK at 5 °C and 20 mg/ml – TAT; 5 mg/ml – TBT; 1.25 mg/ml – TCT; 0.312 mg/ml – TDT; control group – TKT at 37 °C. The occurrence of morphologically abnormal spermatozoa was quantified microscopically at 1000x magnification, and the following abnormal morphological changes were evaluated: SH – separated heads, AC – acrosomal changes, KTF – knob-twisted flagellum, FT – flagellum torso, FB – flagellum ball, LH – large head, SH – small head, PPS – % of physiological spermatozoa and PMC – % of morphological changed spermatozoa. A higher number of normal spermatozoa (PPS) was found in the control group (TK) (84.33%) and the lowest number was detected in the TB group (79.92%). Morphological analysis showed similar occurrence of morphological abnormalities in all groups at 5 °C with no significant differences ($p>0.05$). The highest percentage of normal spermatozoa was found in the control group (TKT) (87.75%). The percentage of abnormal (morphologically changed – PMC) spermatozoa was the highest in the TAT group (18.33%). Significant differences were observed only in one parameter – acrosomal changes, between groups: TAT (5.41%) and TBT (3.25%) ($p<0.05$); TAT (5.41%) and TCT (2.83%) ($p<0.01$); group TAT (5.41%) compared to TDT (2.16%) and control group (TKT; 2.16%) ($p<0.001$). In other observed parameters no significant differences were found, but percentage of other parameters correlated with increasing concentration of trehalose. Results of our study showed that morphological changes of spermatozoa were elevated in case of higher trehalose. Only a few of our results were statistically significant, therefore further research is needed.

Keywords: trehalose, dog, spermatozoa, CASA, morphological change

Acknowledgments: This work was supported by a VEGA project No. 1/0532/11.

THE EFFECT OF RESISTIN ON CRP SECRETION FROM LYMPHOCYTES IN RESPONSE TO INFLAMMATION

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Resisitin, a cytokine which is produced by adipose tissue, takes a part in decreasing insulin sensitivity, endothelial dysfunction, and atherosclerosis. Clinical studies confirmed also that resistinis a moleculewith a pro-inflammatory potential. The aim

of this study was to examine the influence of resistin supplementation on C-reactive protein lymphocytic secretion in piglets during different inflammation states: acute induced by injection of streptozotocin (STZ) and chronic associatedwith obesity. Experiment was carried out on 10 weeks-old piglets (Polish Landrace, females, n=24) kept in standard conditions. Animals were divided into 4 groups: I – control, II – with obesity (chronic inflammation), III – acute inflammation and IV – with obesity and acute inflammation. Peripheral blood lymphocytes (PBL) were isolated from fresh heparinized blood using standard gradient sedimentation technique. Isolated lymphocytes were placed in medium (RPMI 1649, 10% fetal calf serum, L-glutamine, penicillin, streptomycin) and cultured for 72 hours with or without 1mM of resistin. C-reactive protein level was estimated by the enzyme-linked immunosorbent assay (ELISA, Life Diagnostic) in culture supernatant. The obtained results showed that during acute inflammation and obesity CRP secretion level increased by 290.9% and 193.9% compare to the control group (184.65±15 ng/10^5leucocytes, $p<0.05$), respectively. The addition ofresistinenhancedimmune response in obese piglets by increasing CRP secretion (29.2%; $p<0.05$).Based on the results we can confirm that CRP lymphocytic secretion depends on physiological conditions and resistin takes a part in the development of inflammation as a modulatorof the immune systemresponse during metabolic disorders.

Keywords: resistin, C-reactive protein, inflammation.

Acknowledgments: Supported by Grant NN 311 227 138, DS3243/KFiEZ/13 and Quality Grant for PhD Students 2013.

IN VITRO ASSESSMENT OF OCTYLPHENOL IMPACT ON SPERMATOZOA MOTILE PARAMETERS

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In this study we examined the effect of various concentrations of octylphenol (OP; Sigma-Aldrich, St. Louis, USA) (1, 10, 100 and 200 µg/mL) dissolved in 1% ethanol (Sigma-Aldrich, Bratislava, Slovak Republic) on the motile parameters of bovine spermatozoa during cultivation times 0 h, 2 h, 4 h and 6 h. The control spermatozoa (Ctrl) group was cultured with physiological saline solution (sodium chloride 0.9% w/v, Bieffe Medital, Italy). The motility analysis was carried out using a CASA (Computer Assisted Semen Analyzer) system – SpermVision™ program (MiniTűb, Tiefenbach, Germany) with the Olympus BX 51 microscope (Olympus, Tokyo, Japan). The following parameters were evaluated: percentage of motile spermatozoa (motility > 5 µm/s), percentage of progressive motile spermatozoa (motility > 20 µm/s), distance average path (DAP; µm) and velocity average path (VAP; µm/s). Data were statistically analyzed using program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). One-way analysis of variance (ANOVA) and the Dunnett´s multiple comparison test were used for statistical evaluations. The control group (medium without OP) was compared to the experimental groups (exposed to different concentrations of OP). Significant differences ($P<0.001$ and $P<0.05$) between the control group and experimental groups were recorded.Obtained data from our studyshowed a decreased spermatozoa motility and progressive motility in all experimental groups with the addition of OP in comparison to the control group during all time periods. The evaluation of OP influence on the motile parameters DAP and VAP indicated a significant decrease and increase in experimental groups with 1, 10 and 200 µg/mL of OP.In conclusion, our results confirm that octylphenol have the detrimental effect on the rated parameters of spermatozoa motility during short-term cultivation.

Keywords: octylphenol, bovine spermatozoa, motile parameters, CASA system

Acknowledgments: This work was supported by the Scientific Agency of the Slovak Republic VEGA No. 1/0532/11.

THE EFFECT OF HT-2 TOXIN WITH YUCCA (*YUCCA SHIDIGERA*) ON SECRETION OF STEROID HORMONES BY PORCINE OVARIAN GRANULOSA CELLS

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The aim of this *in vitro*study was to examine the secretion of progesterone, testosterone and estradiol by ovarian granulosa cells after addition of HT-2 toxin in combination with yucca.Ovarian granulosa cells were incubated withouth (control) or with treatments at the doses 1, 5 and 50 ng/ml of HT-2 toxin plus yucca50 µg/ml for 48h. Progesterone, testosterone and estradiol were determined by RIA. After addition of 5, 50 ng/ml (but not at 1 ng/ml) of HT-2 toxin with yucca 50 µg/ml the release of progesterone was significantly ($P<0.05$) inhibited. The release of testosterone was significantly ($P<0.05$) inhibited by 1 $ng.ml^{-1}$ (but not at 5 and 50 ng/ml) of HT-2 toxin with 50 µg/ml

of yucca. We observed significant ($P<0.05$) stimulation in estradiol release in all experimental groups. There is a little information about the effect of A-trichothecenes on secretion activity of animal ovarian granulosa cells. The results of our *in vitro* experiments indicate the possible dose-dependent effect of HT-2 toxin with yucca on secretion activity to release steroid hormones.

Keywords: HT-2 toxin, yucca, progesterone, testosterone, estradiol, granulosa cells.

Acknowledgments: The authors are thankful to Romer Labs Division Holding GmbH, Tulln, Austria for providing mycotoxins and colleagues from the Institute for Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra, particularly to Mrs. Katarina Tothova and Ing. Zofia Kuklova for their skillful technical assistance. This work was financially supported by the Ministry of Education of the Slovak Republic project no. 1/0790/11, 1/0084/12, 1/0022/13 and KEGA 030 SPU – 4/2012.