Poster – [A-10-1121-3]
Interaction of adiponectin and its receptors gene expression in adipose tissue with thyroid hormones in experimental hypo and hyperthyroidism in rat
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Abstracts

Materials and methods: Sixty healthy adult male Sprague Dawley rats were randomly divided into three groups, as control (fresh water), hypothyroidism group (methimazole in the drinking water for 42 days, 250 mg/l), and hyperthyroidism group (levothyroxin in the drinking water for 42 days, 12 mg/l). Hypothyroid, hyperthyroid and euthyroid rats were killed after 15, 28 and 42 days of treatment and also two week after treatment cessation. The retroperitoneal (RET) and epididymal (EPI) white adipose tissues were removed completely for mRNA isolation. To evaluate the level of adiponectin gene expression in adipose tissue of different treated animals quantitative real time PCR (qRT-PCR) was performed using qPCR™ Green Master Kit for SYBR Green ™ (Jena Bioscience, Germany). Relative quantification was performed according to the comparative 2-ΔΔCt method. Levels of total T3 and T4 and free T3 (fT3) and free T4 (fT4) were determined with the RIA kits (Immunotech, Radioliv, Czech Republic).

Results: The results indicated that adiponectin mRNA level increased significantly in hyperthyroid rats compared with the normal and hypothyroid rats (P < 0.05). Whereas, adiponectin mRNA level in hypothyroid rats decreased significantly in compared with hyperthyroid and normal rats (P < 0.05). Adiponectin mRNA level had a positive correlation with serum thyroxine (r = 0.861, P = 0.01), triiodothyronine (r = 0.893, P = 0.01), fT3 (r = 0.794, P = 0.01) and fT4 (r = 0.697, P = 0.01). Similar results were found about the gene expression pattern of adiponectin receptors in rat adipose tissue in hypo and hyperthyroidism. In conclusion, gene expression levels of adiponectin and its receptors were increased in hyperthyroidism and decreased in hypothyroidism states.

Keywords: Adiponectin, Adiponectin receptors, Gene expression, Reproductive, Estrous cycle, Pregnancy, Bovine

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Poster – [A-10-1121-4]
Novel gene expression pattern of adiponectin and adiponectin receptors in dominant and nondominant follicles and oocytes screened for their developmental competence based on brilliant cresyl blue staining
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Abstracts

Introduction: Adiponectin, an adipocyte-derived protein, is an important component of the homeostatic mechanisms that regulates body weight and lipid/carbohydrate metabolism. Available experimental data suggest that adiponectin and thyroid hormones share some biological effects but the molecular mechanisms of regulation of adiponectin and its receptors gene expression by thyroid hormones is unknown. The studies described herein were intended to examine the mechanism of regulation of adiponectin gene expression in rat adipose tissue in both hypo and hyper thyroidism.

Materials and methods: Based on estradiol/progesterone ratio, two largest follicles from ovaries collected at a slaughterhouse were classified as NDFs and DFs. In addition the stages of the estrous cycle (follicular or luteal phases) were defined by macroscopic observation of the ovaries and the uterus. Compact cumulus–oocyte complexes were stained with BCB for 90 min. The relative expression of adiponectin, AdipoR1 and AdipoR2 mRNA in theca and cumulus cells and oocytes of different follicles were determined by quantitative real time PCR and qPCR™ Green Master Kit for SYBR Green ™ (Jena Bioscience, Germany). Relative quantification was performed according to the comparative 2-ΔΔCt method. Levels of total T3 and T4 and free T3 (fT3) and free T4 (fT4) were determined with the RIA kits (Immunotech, Radioliv, Czech Republic).

Results: Adiponectin and its receptors genes were cleared expressed higher (P < 0.05) in theca and cumulus cells and oocytes of DFs than those of NDFs during the follicular and luteal phases of bovine estrous cycle. BCB + cumulus–oocyte complexes showed a higher (P < 0.05) expression of adiponectin and its receptors
compared with their BCB– counterparts. Positive correlation ($r = 0.7, P = 0.0048$) was observed between adiponectin mRNA level in ovarian cells of DFs and follicular fluid estradiol concentration in follicular phase. Adiponectin mRNA abundance in ovarian cells of NDFs showed a significant negative correlation with follicular fluid progesterone concentration in follicular phase ($r = -0.73, P = 0.0014$) and luteal phase ($r = -0.81, P = 0.0027$).

**Keywords:** Adiponectin, Adiponectin receptors, Gene expression, Follicles, Competent oocytes

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**Poster – [A-10-1125-1]**

Study of the possible repression for apoptosis in breast cancer vitrified cells (T-47D) in the presence of DMSO as a cryoprotectant

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**Introduction:** Cryobiology is the study of effects of extremely low temperatures on biological systems. It has been reported that cryopreservation induces apoptosis in some vitrified cells. In this study, we examined activation of apoptotic pathway during cryopreservation of T47-D cell line in the presence of DMSO.

**Methods:** T-47D cells were cryopreserved in liquid nitrogen with different concentrations of DMSO. Survival of the cells was assessed using MTT assay in comparison with non-frozen controls. Samples were prepared after thawing and transferred into RPMI culture medium at 37 °C for 24, 48 and 72 h. Then, expression of some important genes involved in apoptotic pathway (e.g. caspase3, bax, bcl2, aif1) were studied using Real-time RT-PCR.

**Results:** The MTT assay results showed that 24 h after thawing, viability of the cells is in the lowest level relative to the fresh cells. Therefore, it was the best time for RNA extraction and performing real-time RT-PCR to determine the possible overexpression of the mentioned apoptotic genes.

**Discussion:** Our results showed that vitrification of T-47D cells has been followed by induction of apoptosis. Therefore, one of the main ways of cryopreservative activity of DMSO might be exerted through its repressive effects on apoptosis in T-47D cells.

**Keywords:** Cryopreservation, Apoptosis, DMSO, T-47D

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**Poster – [A-10-1143-1]**

Compare polymorphisms in the introns 1 and 4 chicken growth hormone gene in the native fowls of Isfahan and Mazandaran

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**Introduction:** The polymorphism in the chicken growth hormone (cGH) gene has been reported. The PCR-RFLP were studied in the various populations of Chinese native chickens and it was suggested that the cGH gene is highly polymorphic in the intron region and the alleles in this region are linked to production traits. The native chicken are a source of genetic variability and genetic conservation in each country. Therefore, the objective of present study was to evaluate the genetic polymorphism in the introns 1 and 4 among native fowls of Iran. The chicken genomic DNA was isolated according to the salting out technique. A fragment with the size of 776 bp from the intron 1 and a fragment with the size of 1170 bp from the intron 4 were amplified using two pairs of specific primers and polymerase chain reaction (PCR). The PCR products were digested withMspI restriction enzyme and then were analyzed on 2.5% agarose gel. The allelic frequency of intron 1 locus for M, N and O alleles in Isfahan native fowls was 0.60, 0.21 and 0.19, respectively and in Mazandaran populations was 0.28, 0.05 and 0.67 respectively. The allelic frequency of intron 4 for A, B and C alleles in Isfahan native fowls populations were 0.28, 0.31 and 0.41 respectively and in Mazandaran population were 0.37, 0.24 and 0.37 respectively. Also D and E alleles were observed in Mazandaran population solely with 0.01 frequency for each allele. The result of current study indicated that the introns 1 and 4 of cGH is relatively highly polymorphic in Isfahan and Mazandaran native fowls.

**Keywords:** cGH, Polymorphism, Iranian native fowls, PCR-RFLP

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