matrixscience.com). Identified proteins were submitted to GO Retriever (http://www.agbase.msstate.edu/) to obtain the GO annotations. Gene ontology derived protein names were submitted to STRING database for protein interaction.

Results: In order to identify the protein for quantification, previously obtained data from Progenesis Same spots software, MS-based quantitative proteomic strategy has been used to compare the proteomes of treated and untreated cells of Hep3B cells. ANOVA test result performed by Progenesis Software was determined to analyze the significant differences in abundant proteins between treated and untreated group ($P<0.05$). Out of 180 spots, 7 spots showed significantly difference and highest number of fold (≥ 2 fold and $p<0.05$). MASCOT derived 6 differentially expressed proteins out of 12 abundant proteins were subjected to GO classification via the Panther Classification System database. In order to predict the protein interaction between obtained differentially expressed protein, STRING database with medium confidence were performed.

Conclusions: Proteomic studies for the identification of proteins aberrantly expressed in cancer cells can potentially be used to develop new diagnostic, prognostic or therapeutic targets. Identification of differentially expressed abundant protein by MALDI-TOF/MS analysis using the MASCOT search engine and the Swiss Prot database will enlarge the category wise quantitative studies in Hep3B cancer treated cells or any other cancer types. The protein interaction between the expressed proteins would suggest their regulatory signaling network and bio-marker function.

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Immunomodulatory Properties of Equine Mesenchymal Stem Cells in Equine Peripheral Blood Mononuclear Cells Stimulated with Mitogen

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Introduction: Mesenchymal stem cells (MSCs) are multipotent cells that can be differentiating into several cells, such as bone, cartilage, tendon, muscle and adipose tissues. The use of MSCs has been the focus of attention in clinical practice to aid bone repair and tendon healing in horses. Recently, it has been reported that MSCs have immunomodulatory properties. In addition, MSCs can suppress immune cells such as T, B, NK, and dendritic cells. The mechanisms of immunomodulatory effects by MSCs are currently unclear. However, it has been reported that the immunomodulatory effects of MSCs partly depend on the secretion of anti- or pro-inflammatory cytokines, including pro-inflammatory factors such as interleukin-1beta (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), and interferon gamma (IFN-γ) and anti-inflammatory factors such as interleukin-10 (IL-10). In this study, we investigated immunomodulatory properties under co-culturing condition of equine adipose tissue-derived MSCs (eAD-MSCs) with equine peripheral blood mononuclear cells (ePBMCs).

Materials and Methods: The eAD-MSCs isolated from tissues were collected from racehorse tail head. The eAD-MSCs were cultured in DMEM-low complete medium. The expression levels of molecules associated with self-renewal, cell surface markers, and differentiation potentials at passage 3 in these cells were examined. Blood samples from racehorse were collected and ePBMCs were isolated using Ficoll-Paque density gradient centrifugation. The eAD-MSCs (4×10^5 cells/well) were plated on the inner transwell membrane (1 μm pore size) and co-cultured with ePBMCs (2×10^6 cells/well) stimulated with mitogen (LPS 1 μg/mL or ConA 5 μg/mL). After co-culturing for 72 hr, the cell culture supernatant was collected. To determine the amount of immunomodulatory cytokines in co-cultured supernatant, commercial equine ELISA kit (R&D Systems) was used according to the manufacturer's instructions.

Results: To examine the immunomodulatory effects of eAD-MSCs under co-culturing condition, ePBMCs were cultured with eAD-MSCs and stimulated with LPS (1 μg/mL) and ConA (5 μg/mL). Our results revealed that the expression levels of pro-inflammatory cytokines (IL-1β, IL-2, IL-4 and IFN-γ) were significantly ($p < 0.05$) increased in LPS-stimulated ePBMCs when compared to those in LPS-unstimulated ePBMCs. Our results also revealed that the expression levels of pro-inflammatory cytokines (IL-1β, IL-2, IL-4 and IFN-γ) were significantly ($p < 0.05$) decreased by eAD-MSCs while the level of anti-inflammatory factor (IL-10) was increased by eAD-MSCs. In addition, the expression levels of pro-inflammatory cytokines (IL-1β, IL-2 and IFN-γ) were significantly ($p < 0.05$) increased in ConA-stimulated ePBMCs when compared to those in ConA-unstimulated ePBMCs. These levels were decreased by eAD-MSCs.

Conclusions: MSCs can modulate the immune system in

part through secreting cytokines and growth factors. Our results showed that the immunomodulatory factors were secreted by eAD-MSCs under co-culturing of eAD-MSCs with ePBMCs. Further study is needed to demonstrate the detailed mechanisms involved in the immunomodulatory effects of eAD-MSCs.

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Lysophosphatidic acid (LPA) stimulates porcine oocytes Maturation and embryonic development *In Vitro*

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Introduction: The lysophosphatidic acid (LPA) is a signaling molecule derived from phospholipid known to have biological activities such as stimulating cell proliferation, differentiation and migration. In this study, we examined the effect of LPA on porcine oocytes during *in vitro* maturation and subsequent embryonic development following PA and IVF.

Materials and Methods: We investigated nuclear maturation, intracellular gluthatione (GSH), reactive oxygen species(ROS) levels analysis, gene expression analysis by Real-time PCR, protein analysis by western blotting and ensuing embryo development after PA and IVF. Each concentration (0, 10, 30, and 60 μM) of LPA was supplemented in maturation medium during *in vitro* maturation. Data were analyzed by ANOVA followed by Duncan using SPSS (Statistical Package for Social Science) mean ± SEM.

Results: After 44 h of IVM, the 30 μM LPA treated group showed significant ($P< 0.05$) increased in nuclear maturation (90.31%)compared with the 0 (control), 10 and 60 μM LPA treated groups (82.50%, 86.22%and 86.72%, respectively). The 30 μM LPA treated group exhibited a significant($P< 0.05$) increase in intracellular GSH levels and decrease in intracellular ROS levels compared with other LPA treated groups. Oocytes matured with 30 μM LPA during IVM had significantly ($P< 0.05$) higher cleavage rates after PA (81.51%)than other LPA treated groups (74.19%, 77.53% and 74.63%, respectively). The blastocyst formation rates, also, 30 μM LPA treated group showed significantly($P< 0.05$) higher (62.04%) than 0, 10 and 60 μM LPA treated groups (50.03%,55.02% and 57.09%, respectively). The IVF embryonic competence, 30 μM LPA treated group had significantly ($P< 0.05$) higher cleavage rates (70.58%)than other LPA treated groups (63.83%, 67.76% and 67.07%, respectively).Likewise, the blastocyst formation rates showed significantly ($P< 0.05$)higher in 30 μM LPA treated group (37.87%) than 0, 10 and 60 μM LPA treated groups (31.07%, 33.93% and 33.97%, respectively). In the case of oocytes and cumulus cells gene expression of apoptotic gene (Caspase-3) in 10 and 30 μM LPA has significantly decreased. In addition uPA and uPAR was specifically expressed in cumulus cells was significantly increased at 30 μM LPA treated group. In addition, LPA improves activities of p38 MAPK by phosphorylation.

Conclusions: In conclusion, the treatment with 30 μM LPA during IVM improved the developmental potential of PA and IVF porcine embryos by increasing the intracellular GSH level, thereby decreasing the intracellular ROS level during oocyte maturation. Also, improves activities of cumulus cells p38 MAPK, induces the expansion of cumulus cells and promotes the maturation of oocytes.

Keywords: porcine, oocyte, *in vitro* maturation, LPA, pre-implantation development, PA, IVF

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Pectolinarigenin Induce Apoptosis on Gastric Cancer Cells

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