World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:11, No:03, 2017

Potential Serological Biomarker for Early Detection of Pregnancy in Cows

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Abstract : Pregnancy is a complex process which includes series of events such as fertilization, formation of blastocyst, implantation of embryo, placental formation and development of fetus. The success of these events depends on various interactions which are synchronized by endocrine interaction between a receptive dam and competent embryo. These interactions lead to change in expression of hormones and proteins. But till date no protein biomarker is available which can be used to detect successful completion of these events. We employed quantitative proteomics approach to develop putative serological biomarker which has diagnostic applicability for early detection of pregnancy in cows. For this study, sera were collected from control (non-pregnant, n=6) and pregnant animals on successive days of pregnancy (7, 19, 45, n=6). The sera were subjected to depletion for removal of albumin using Norgen depletion kit. The tryptic peptides were labeled with iTRAQ. The peptides were pooled and fractionated using bRPLC over 80 min gradient. Then 12 fractions were injected to nLC for identification and quantitation in DDA mode using ESI. Identification using Mascot search revealed 2056 proteins out of which 352 proteins were differentially expressed. Twenty proteins were upregulated and twelve proteins were down-regulated with fold change > 1.5 and < 0.6 respectively ($p < 0.05$). The gene ontology studies of DEPs using Panther software revealed that majority of proteins are actively involved in catalytic activities, binding and enzyme regulatory activities. The DEP'S such as NF2, MAPK, GRIPI, UGT1A1, PARP, CD68 were further subjected to pathway analysis using KEGG and Cytoscape plugin Cluego that showed involvement of proteins in successful implantation, maintenance of pluripotency, regulation of luteal function, differentiation of endometrial macrophages, protection from oxidative stress and developmental pathways such as Hippo. Further efforts are continuing for targeted proteomics, western blot to validate potential biomarkers and development of diagnostic kit for early pregnancy diagnosis in cows.

Keywords : bRPLC, Cluego, ESI, iTRAQ, KEGG, Panther
Conference Title : ICABABP 2017 : International Conference on Animal Biotechnology and Animal Breeding Programs
Conference Location : Miami, United States
Conference Dates : March 09-10, 2017