ISAG 2021
VIRTUAL CONFERENCE
INTERNATIONAL SOCIETY FOR ANIMAL GENETICS
JULY 26–30, 2021

ABSTRACTS

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genes (DEGs) were extracted by FDR <0.05, |$\log_2$ fold change (FC)| ≥ 1. As a result, 7,623 significant DEGs were detected (upregulated genes 2,692, downregulated genes 4,931) and the genes were partitioned into 3 clusters according to the expression pattern. Cluster 1 and 2 (1,222 and 1,146 genes) showed overall physiological changes through the estrous cycle. Cluster 1 majorly involved in PI3K-Akt signaling and steroid hormone biosynthesis pathways and Cluster 2 were involved ECM-receptor interaction and protein digestion. The expression pattern of Cluster 3 was uniquely downregulated at luteal phase and network was constructed with 1,000 genes. KEGG pathway enrichment analyses revealed that DEGs in Cluster 3 were associated with cell cycle, calcium signaling, oocyte meiosis etc. As a result of gene set enrichment analysis (GSEA) networking, calcium signaling pathway and oocyte meiosis were also significantly observed in the luteal phase. Calcium signaling pathway which calcium causes sperm hyperactivity by progesterone and helps oocyte transport through smooth muscle contraction and oocyte meiosis affect oocyte migration and fertilization. As genes directly related to fertilization, the most important role in the oviduct, were relatively downregulated in the luteal phase, it can be inferred that genes are relatively upregulated in the follicular phase and play a prominent role in reproductive tissues during estrous cycle.

Key Words: pigs and related species, functional genomics, RNA-seq, gene expression, reproduction

P336 Time serial ovarian transcriptome analysis for entire porcine estrous cycle reveals changes of steroid metabolism and corpus luteum development. Y. Park*, Y.-B. Park, S.-W. Lim, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

The estrous cycle (estrus, metestrus, diestrus, and proestrus) is a physiological process that occurs in most mammalian females under the effect of reproductive hormones. This cycle affects reproduction and causes many changes, especially in the reproductive organs. Among them, an ovary is an important place where ovulation, luteinization, CL development, and luteolysis take place. For a more in-depth study of the dynamic changes in gene expression, the transcriptome of the porcine ovary was observed in the estrous cycle at intervals of 3 d from d 0 to d 18. A total of 4,414 DEGs were identified at 7 time points of the estrous cycle, and these were classified into 3 clusters according to the transcriptome expression pattern. During diestrus, the expression of the transcriptome increased rapidly, and cluster 1 was upregulated at that period, whereas clusters 2 and 3 tended to be downregulated. We performed functional analysis of the genes included in each cluster, selected KEGG pathways related to the gene ontology (GO) terms, and identified significant genes among them. In cluster 1, GO results were found that included terms such as intestinal absorption and sterol biosynthesis, and based on this, steroid biosynthesis was selected for the significant KEGG pathway. In cluster 2, cytokine-cytokine receptor interaction was chosen as important KEGG pathways based on GO outcomes such as neutrophil chemotaxis and regulation of timing of cell differentiation. Finally, morphogenesis and embryo development-related terms were shown in cluster 3, and the hedgehog signaling pathway was selected. Our study exhibited the dynamic changes and a comprehensive understanding of the porcine ovary during the estrous cycle through DEG profiling and transcriptome analysis. Especially, we found several genes that were affected to hedgehog signaling pathway and consequently, this suggests that genes that influence embryonic development during the diestrus are expressed in the ovary. Further studies should be conducted with every estrous cycle to understand the mechanism of the porcine ovary.

Key Words: pigs and related species, functional genomics, gene expression, RNA-seq, reproduction

P337 Overview of long noncoding RNA and mRNA annotation throughout swine estrous cycle in reproductive tissues. Y.-B. Park*, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Long noncoding (lnc) RNAs were reported to regulate target genes at transcriptional, posttranscriptional, and posttranslational regulation levels in a wide variety of species. The reproductive traits of porcine are very closely related to economics among economic traits, and it has been understood through various preliminary studies that several reproductive tissues are involved in a complex manner. However, unlike mRNA, lncRNA annotation has not been sufficiently performed, and understanding at the level of protein-coding gene has limitations in determining the mechanism of reproductive traits of porcine. Therefore, we investigated lncRNAs of porcine ovary, oviduct and endometrium at d 0, 3, 6, 9, 12, 15, or 18 of the estrous cycle. We focused on analyzing the expression patterns of mRNA and lncRNA and finding the key lncRNA through network analysis. The patterns of differentially expressed mRNA and differentially expressed lncRNA were similar, and we identified 10 key lncRNAs. Our analysis suggests lncRNAs and mRNAs that are differentially expressed according to the porcine reproductive tissue and estrus cycle and are involved in the reproductive trait mechanism by regulating pathways such as steroid hormone synthesis.

Key Words: pigs and related species, bioinformatics, noncoding RNA, RNA-seq, gene expression

P338 Signature of stress-related characteristics according to changes in pig breeding condition through transcriptome analysis. S.-W. Lim*, B. Lim, and J.-M. Kim, *Functional Genomics and Bioinformatics Lab, Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea.*

Pig breeding condition, such as density-stress, is one of the important factors in terms of pig productivity. However, the molecular mechanisms for the level of whole-genome expression depending on the pig breeding condition has not been well studied. The purpose of this study is to identify functional mechanisms according to changes in pig breeding condition through transcriptome analysis. In this study, we accepted samples from 3 conditions (control, welfare and density) and observed their transcriptomic changes using the RNA-seq method. Gene alignment and gene transfer format of the entire pig genome was annotated using Sus scrofa 11.1.102. Whole gene expression profiling was performed using a general linear model using edgeR of R package. The differentially expressed genes (DEGs) were extracted by the *P*-value <0.01 with absolutely expressed for double changes to each comparison group. As a result, for each group genes were identified into the DEGs (welfare vs. control; a total of 109 genes, downregulated genes 62 and upregulated genes 47, density vs. control; a total of 199 genes, downregulated genes 126 and upregulated genes 73, welfare vs. density; a total of 135 genes, downregulated genes 55 and upregulated genes 80). Gene Ontology (GO) functional enrichment analyses distinguished the DEGs primarily associated with metabolism, signaling molecules and circulatory systems. KEGG pathway enrichment analyses revealed the DEGs primarily associated with oxidative stress signaling and immune signaling. Therefore, the biological process in breeding condition suggests that the expression of stress-related genes through metabolic and immune signals were high. We believe that further research would be required to understand more precise mechanisms.

Key Words: pigs and related species, functional genomics, RNA-seq, animal welfare, behavior

P339 Muscle proteomics of preweaning piglets from sows fed diets with extreme ω-6/ω-3 fatty acid ratios. Y. Manaig*[1,3], A. Agazzi[3], S. Panseri[3], G. Tedeschi[3], J. Folch[1,2], A. Sanchez[1,2], and G. Savoini[3], [1]*Universitat Autònoma de Barcelona, Barcelona, Spain,* [2]*Centre for Research*

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An optimal ratio of omega-6 and omega-3 polyunsaturated fatty acids (PUFA) plays an essential role to maintain metabolic modulations and homeostasis, mainly due to their contrasting inflammatory functions. At present, there are a few studies on how sow nutrition directly affects piglet muscle deposition, especially before weaning. The study was conducted to determine on how the sow's milk, fed with extreme ω-6/ω-3 FA ratio diets, directly affects the expression profiles of genes, abundance of proteins, and their biological pathways. A total of 8 multiparous sows were used and divided between 2 dietary treatments with ω-6/ω-3 FA ratios of 13 (SOY) and 4 (LIN), mainly derived from soybean and linseed oil, respectively. Piglets were nourished only with sow's milk during lactation. At the end of lactation period, a total of 24 longissimus dorsi muscle from piglets (12 males and 12 females) were collected and subjected to proteomics analysis based on nano liquid chromatography coupled to high-resolution tandem mass spectrometry (nLC-HRMS) and FA profiles were determined using GC-MS. Of the 412 proteins identified by Proteome Discoverer 2.5 software, 4 proteins (haptoglobin, phosohoglycerate kinase-2, interferon-induced GTP-binding protein Mx2, and prophenin and tritrpticin precursor) were over-abundant ($P < 0.05$) in SOY compared with LIN. Enrichment analysis of identified proteins for gene ontologies (GO) and pathways ($P < 0.05$) showed annotation on GO terms related to fatty acid β-oxidation, high-density and very low density lipoprotein particle assembly, citrate cycle, and glycolysis/gluconeogenesis. These pathways are involved in glucose and lipid metabolism and are mostly regulated by FAs. Moreover, the observed over-abundancy of haptoglobin, an acute phase protein, in sows fed the SOY diet could be related to the proinflammatory role of ω-6 FA. Fat deposition on muscle showed a great resemblance to the diets – 15.3 vs 8.6, SOY vs LIN. A separate in-depth transcriptomics analysis are ongoing and thus will further elucidate the effect of extreme ω-6/ω-3 FA ratio on the expression profiles of genes and microRNAs on piglet muscle.

Key Words: piglet, sow milk, fatty acids, proteomics, muscle

P340 Structural genetic basis of differential gene expression in loin muscle of Iberian pigs. A. López-García*[1], R. Peiro[1], M. Muñoz[1], C. García-Contreras[1], M. Vázquez-Gómez[2], B. Isabel[3], A. Rey[3], A. González-Bulnes[1], and C. Óvilo[1], [1]*INIA (CSIC), Madrid, Spain,* [2]*INSERM (UPS), Paris, France,* [3]*UCM, Madrid, Spain.*

Iberian pig production has acquired growing importance in recent years, due to the increasing demand for high-quality dry-cured products. However, farm productivity is lower compared with other commercial breeds, mainly due to the low prolificacy and uterine capacity of Iberian pigs. Variation in prenatal growth and birth weight can increase this problem, as prenatal muscle development and intramuscular adipogenesis are determinant in postnatal growth. Recent studies of prenatal muscle transcriptome in Iberian pigs have shed light on the molecular basis of the differences in prenatal growth between Iberian and crossbred pigs. Our next step focuses on the genetic basis of these gene expression differences by genetic variant discovery. Muscle RNA-seq data obtained from purebred and crossbred Iberian fetuses at d 77th of pregnancy were used to identify allelic variants between breeds and between high and low weight fetuses. Variant calling was performed using GATK pipeline. Variant filtering was performed, by multiple quality attributes, including minor allele frequency and missingness per variant. 197,732 polymorphisms were detected, and Variant Effect Predictor (VEP) was then used to annotate variants. We kept variants associated with genes reported as differentially expressed (DE) between Iberian and crossbred fetuses (645 genes) or between high and low weight fetuses (35 DE genes for Iberian and 60 for crossbred). PLINK toolset was used to recalculate allele frequencies. Final data set included 8,278 SNPs (14.57 average SNPs per gene, 24% missense variants and 13.5% deleterious), and 866 indels (3.10 average indels per gene, 3.5% coding alteration indels) associated with DE genes. Genes with the highest expression differences between purebred and crossbred fetuses were studied in depth. For instance, we found interesting variants associated with FOXO3 (24 SNPs, 1 indel, 1 missense), LEPR (27 SNPs, 2 indels, 5 missense) or APOD (1 SNP, deleterious). The work done provides useful SNP data and functional information for future association studies in strong candidate genes for early development-related traits in pigs.

Key Words: Iberian, RNA-seq, variant, SNP

P341 *AGPAT5* gene influences fat content and composition in pigs. E. Molinero*, R. N. Pena, J. Estany, and R. Ros-Freixedes, *Departamento de Ciencia Animal, Universidad de Lleida – AGROTECNIO-CERCA Center, Lleida, Spain.*

The 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs) are enzymes that catalyze the conversion of lysophosphatidic acid to phosphatidic acid. Phosphatidic acid is a precursor of triacylglycerol (the main fat reservoir in mammals) and various glycerophospholipids, as well as a signaling molecule involved in multiple regulatory processes. Therefore, we investigated the role of *AGPAT5* gene variants on fat content and fatty acid composition in pigs. We used sequence data to search for variants in the *AGPAT5* gene. A single nucleotide polymorphism in exon 6 (rs196952262, A>G), which produces a missense mutation, was selected as a tag variant for the 11 identified variants in the *AGPAT5* gene that segregate at a frequency higher than 0.05. The effect of this variant was validated in muscle, subcutaneous fat and liver samples of 1,073 pigs from a Duroc line genotyped using a high-resolution melt protocol. The A allele showed a positive additive effect for intramuscular fat (+0.29% ± 0.09 in the *gluteus medius* muscle and +0.24% ± 0.09 in the *longissimus* muscle, $P < 0.01$) and backfat thickness (+0.48 mm ± 0.20 at 180 d of age and +0.60 mm ± 0.24 at slaughter, $P < 0.05$). We also observed a significant effect on fat composition, resulting in more monounsaturated fatty acids and less polyunsaturated fatty acids as a consequence of the increased intramuscular fat content. The increase of monounsaturated fatty acids is desirable, because it is positively related with sensorial, technological and nutritional attributes of pork. However, only the arachidonic acid, which is particularly abundant in membrane phospholipids, was associated with the A allele after accounting for intramuscular fat content (−0.04% ± 0.02 in *gluteus medius* muscle, $P < 0.05$). We showed that the *AGPAT5* gene is involved in fatty acid deposition in pigs and that variations in the gene sequence affect fat content (backfat thickness and intramuscular fat), with specific effects on the arachidonic acid content.

Key Words: pigs and related species, animal breeding, DNA sequencing, fat/lipid, genomic selection

P342 Functional variant identification of cis-eQTL associated with pig *NUDT7* gene and its association analysis with meat color traits. X. Xu*[1,2], L. Liu[1,2], L. Liu[1,2], T. Ma[1,2], Z. Zheng[1,2], and X. Xu[1,2], [1]*Huazhong Agricultural University, Huazhong Agricultural University, Wuhan, Hubei Province, China,* [2]*Key Lab of Swine Genetics and Breeding of Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University, Wuhan, Hubei Province, China.*

Pork color is an important indicator affecting consumption choice, but the candidate genes and molecular markers that regulate pork color are rarely reported. M. Taniguchi et al. localized the QTL affecting pork color (measured by heme content) on chromosome 6, and experimentally proved that the overexpression of *NUDT7* gene may lead to downregulation of heme biosynthesis in skeletal muscle. Our previous cis-eQTL study in skeletal muscle identified a significant signal associated with the expression level of *NUDT7*. Based on the relative difference of FPKM values, individuals with extreme high values and extreme low values were found to have significant differences in the expression levels of exons 3 and 4 and transcripts. Amplification and sequencing results show that the high group of rs326029010 is GA heterozygous, and the low group is GG homozygous. Furthermore, we carried out PCR-RFLP analysis on 416 individuals, and association analysis showed that the polymorphism of