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166 Primordial germ cell-based biobanking of Hungarian indigenous chicken breeds. Bence Lazar*[1], Roland Tóth[1], Alexandra Nagy[2], Mahek Anand[3], Krisztina Liptói[4], Eszter Patakiné Várkonyi[4], and Elen Gócza[1], *[1]NARIC, ABC, Godollo, Hungary, [2]Veterinary Science University, Budapest, Hungary, [3]SZIU, Doctoral School of Animal Husbandry Science, Godollo, Hungary, [4]Research Centre for Farm Animal Gene Conservation, Godollo, Hungary.*

Nowadays most of the economically important or indigenous chicken breeds are preserved in in situ populations, which poses numerous risks, such as epidemics (e.g., avian influenza), environmental disasters, inbreeding and management failure. Therefore, germplasm preservation in poultry is a high priority, although it is faced with difficulties. Embryo cryopreservation is not yet established and semen conservation lacks the ability to preserve the W chromosome and the mitochondrial DNA. Currently, the most promising solution is the cryopreservation of primordial germ cells (PGCs). In this study, by utilizing the unique nature and accessibility of PGCs (they use the vascular system during their migration toward the genital ridge), biobanks for 2 of the indigenous Hungarian chicken breeds (Partridge color, Transylvanian Naked Neck) were established to conserve their genetic resources. Blood samples were collected from each embryo individually, and then the isolated blood, containing the PGCs, was cultured in a PGC selective medium for 3 weeks. After that, PGC samples were collected for DNA, RNA isolation and immunohistochemistry to characterize the quality of the cultured lines. As a next step, parallel vials were frozen from each PGC line, and they have been stored in liquid nitrogen since then. To evaluate the freezing process and to prove the functional integrity and migration ability of PGCs after long-term in vitro cultivation, some of the vials were thawed and the cells were injected into recipient embryos. The cells were labeled with an in vivo fluorescent dye, thus the migration of the injected cells was followed toward the developing gonads, and the ratio of the colonization was analyzed. During the study, 26 individual PGC lines from Transylvanian Naked Neck chicken and 21 lines from Partridge color Hungarian chicken were established with a derivation rate of 46,4% and 31,1% respectively. The PGC lines were frozen and then successfully thawed with a cell viability of around 50% in both lines. The preserved cells were capable of colonizing the gonads of the recipient embryos. This is the first demonstration of a successful PGC-based cryopreservation system applied to Hungarian indigenous breeds and the first initiative in Hungary to establish a biobank based on PGCs.

Key Words: germplasm, cryopreservation, biobanking, primordial germ cells (PGCs), Hungarian indigenous breeds

167 Differences in jejunal gene expression of two chicken lines divergently selected for antibody response to sheep red blood cells. Shelly Nolin* and Christopher Ashwell, *North Carolina State University, Raleigh, NC.*

For over 30 generations 2 lines of White Leghorn chickens have been undergoing continuous divergent selection for high (HAS) or low (LAS) antibody titer to sheep red blood cells (SRBCs) at 5 d post injection. This has been a well-utilized model for immunology and genetic trials, and many differences between the lines have been observed in terms of performance and response to diseases. However, little has been done to study native molecular differences between lines, so the purpose of this experiment was to examine differences in gene expression in the jejunum of non-SRBC injected birds. Eggs from both lines were obtained from Virginia Polytechnic Institute and State University (Blacksburg, VA) and co-incubated until hatch, after which all chickens were housed and raised together in mixed cages. At 46 d of age, 6 chickens from each line were euthanized and jejunum samples were collected for RNA isolation. RNA was isolated using the RNeasy kit by Qiagen and then sent to the NC State University Genomics Sciences Laboratory for library preparation and sequencing on the Illumina HiSeq 2500 sequencer. RNA sequence data were analyzed using NGS RNA analysis tools, TopHat and Cufflinks (statistical significance threshold of $q < 0.05$) available at galaxy.org. Significant differences in gene expression were observed between lines with over 4 times as many genes upregulated in HAS as compared with LAS. Not surprisingly, many of the upregulated HAS genes are involved with immune response, particularly interferon signaling and antigen processing. Genes upregulated in LAS largely involve fatty acid transport and cell membrane integrity. Understanding native gene expression provides insight into the ways in which energy resources are allocated within an organism. It has previously been reported that HAS is slower growing with delayed performance compared with LAS, and the increased immune system related gene expression could indicate priority resource allocations to these systems over growth and performance, further supporting those observations. More research is needed to better understand the effects of genetic selection on physiology to optimize genetic selection for performance and health.

Key Words: gene expression, RNA sequencing, antibody response, genetic selection, HAS/LAS

168 Time course transcriptional analysis of response to Newcastle disease virus infection and heat stress in two genetically distinct inbred chicken lines in Harderian gland tissue. Perot Saelao*[1], Ying Wang[1], Ali Nazmi[1], Rodrigo Gallardo[1], David Bunn[1], Terra Kelly[1], Susan Lamont[2], and Huaijun Zhou[1], *[1]University of California–Davis, Davis, CA, [2]Iowa State University, Ames, IA.*

Biotic and abiotic factors can influence the production potential of both small and large scale poultry flocks. Newcastle disease virus (NDV) and heat stress are 2 major limiting factors that can disrupt physiological processes, and alter the hosts' ability to mount an effective immune response, which could result in significant economic losses. RNA sequencing enables a comprehensive profiling of the activities of genes at the transcriptional level and evaluates the molecular mechanism of disease response during heat stress. The objective was to identify functionally relevant genes and biological pathways associated with response to NDV infection while under heat stress. Two genetically distinct inbred chicken lines (Fayoumi and Leghorn) were heat treated continuously at 35C on d 14, then challenged with NDV or PBS at 21 d old. At 2 d post-infection (DPI), 6 DPI, and 10DPI, Harderian gland tissue was collected (4 individuals per line per treatment) and total RNA was then extracted and used to conduct gene expression analysis with an FDR <0.1. Analysis found 9, 38, and 2,867 differentially expressed genes (DEG) for Fayoumi at 2, 6, and 10 DPI with only the PRKCD gene shared across all time points. Leghorn had 18, 3,993, and 50 DEG at 2, 6, and 10 DPI, respectively. Pathway analysis of the 2 lines found an enrichment of immune related pathways in the Fayoumi data set with a significant enrichment in the AGE/RAGE pathway, which is involved in innate immunity and regulating heat stress. These genes and pathways offer potential targets for further investigation into their roles in responding to infection during heat stress. Funding provided by US Agency for International Development (USAID) AID-OAA-A-13-00080.

Key Words: host immune response, genomics, RNA-Seq