



번식 및 생리

Effects of circadian rhythm-based vaccination timing on antigen-specific fecal IgA responses in broiler chickens

Seonju Lee¹, Eun Bae Kim^{1,2,*}

¹Department of Applied Animal Science, College of Animal Life Sciences, Kangwon National University, Chuncheon 24341, Korea

²Institute of Animal Life Science, Kangwon National University, Chuncheon 24341, Korea

Recent studies highlight the role of circadian rhythms in modulating immune function; however, vaccination strategies incorporating circadian rhythms remain limited in the poultry industry. Avian immune responses exhibit circadian variation driven by melatonin and metabolic activity. Therefore, understanding antibody dynamics associated with circadian rhythms is essential for optimizing vaccine efficacy. This study evaluated how vaccination timing in relation to circadian rhythms modulates immunogenicity in chickens. Chickens were divided into morning (10:30) and afternoon (18:00) vaccination groups, and each group received the same dose of antigen via oral administration twice at 2-week intervals (four total administrations). Fecal samples were collected one week after the final immunization, and antigen-specific IgA titers were measured using sandwich ELISA. The results showed that, despite identical antigen dosage and administration frequency, significant differences in fecal antigen-specific IgA titers were observed depending on the time of vaccination. Notably, the morning group exhibited significantly higher antibody titers than the afternoon group (Paired *t*-test, $p < 0.001$). These findings demonstrate that circadian rhythm-based vaccination timing significantly influences fecal IgA responses and suggest that such strategies provide a practical approach to enhance vaccine immunogenicity within the livestock industry. Supported by NRF/MSIT (RS-2024-00344849).

Key words : circadian rhythm, vaccination, antigen-specific IgA, oral administration

Prolonged farrowing and impaired nursing behaviour are associated with gut microbial alterations in sows with large litters

Yujin Jung¹, Woohee Chung¹, Jua Woo¹, Juho Lee^{1,2}, Cheolju Park¹, Minseok Kim¹, Jinhyeon Yun^{1,*}

¹Department of Animal Science and Bioindustry, Chonnam National University, Gwangju, Korea

²Department of Agricultural and Food Sciences, University of Bologna, 40127 Bologna, Italy

Increased litter size in modern pig production has been associated with prolonged farrowing and impaired maternal behaviour. Given the role of the gut microbiota in regulating neuroendocrine and behavioural processes, this study investigated whether maternal dysfunction associated with large litter size is related to alterations in gut microbial composition. A total of 22 primiparous sows (Landrace × Large White) were classified into normal litter (NL, litter size 11.5 ± 1.0 , range 7-14, $n = 8$) and large litter (LL, litter size 15.6 ± 0.3 , range 15-18, $n = 14$) groups. Maternal traits, including colostral prolactin levels and nursing behaviour, were evaluated. Faecal microbiota was analysed using full-length 16S rRNA sequencing on the PacBio platform. Taxa more abundant in the NL group, particularly *Lactobacillus* spp. (e.g., *L. reuteri* and *L. gasseri*), were positively correlated with colostral prolactin levels ($r = 0.47$, $p < 0.05$) and nursing frequency ($r = 0.48$, $p < 0.05$). In contrast, taxa more abundant in the LL group, including *Roseburia* and *Campylobacter*-related taxa, were positively correlated with farrowing duration ($r = 0.43$ – 0.46 , $p < 0.05$). In addition, *Campylobacter coli* showed a tendency for a negative correlation with nursing duration ($r = -0.40$, $p = 0.06$). These results indicate that prolonged farrowing and impaired nursing behaviour in large litter sows are associated with distinct gut microbial profiles. The correlation results further suggest a potential role of microbiota-neuroendocrine interaction in hyperprolific sows.

Key words : farrowing duration, gut microbiota, hyperprolific sow, maternal behaviour, maternal hormone

Immortalization of Hanwoo reproductive cells for heat stress *in vitro* research

Ismail Shaleh^{1,2,*}, Sung Woo Kim³, Tae Sub Park¹, Joonghoon Park^{1,4}

¹Graduate School of Int'l Agriculture Technology, Green Bio Science & Technology,
Seoul National University, Korea

²Department of Biology, Faculty of Mathematics and Natural Science, IPB University, Indonesia

³Hanwoo Research Institute, National Institute of Animal Science, Korea

⁴Department of Agricultural Biotechnology, Research Institute of Agriculture and Life Science,
Seoul National University, Korea

Substantial increase in global temperature induces heat stress (HS) in livestock animals particularly dysregulating cattle reproductive capacity leading to economic loss. Development of *in vitro* models in cattle reproduction is a critical process to unveil molecular basis of HS destructive effects and to elaborate treatments for mitigation. Therefore, this research proposes to immortalize reproductive cells isolated from Hanwoo, native Korean cattle that are susceptible to HS, and to examine HS-associated response using molecular markers and omics techniques. This research successfully establishes three immortalized Hanwoo cells i.e. cumulus cells (CC), oviduct epithelial cells (OEC), and endometrium epithelial cells (EEC) by integrating SV40T Ag into genome using *piggy bac* transfection system confirmed by PCR. Specific biomarkers showed high positivity score (> 98%), particularly on CC and OEC cell populations. In general, HS treatment at 41.5°C for 4 h triggered intracellular reactive oxygen species (ROS) accumulation in all three cell lines. Nevertheless, delayed response of oxidative stress genes (*SOD1*, *CAT*, *GPXI*) were observed with significant upregulation (2–3x foldchange, $p < 0.05$) peaking at 24 h HS treatment. Besides, rapid increase of HSP70 protein chaperone (~10x foldchange, $p < 0.05$) was detected under the 4 h HS peaking at 12 h HS treatments, while ER-stress marker BiP/GRP78 protein showed various upregulation time points among different cells. Prolonged response of the stress markers was also presented during recovery period. Additionally, transcriptomics and proteomics profile showed that HS treatment altered more than 550 and 250 differentially expressed genes (DEG) and proteins (DEP), respectively. Based on this gene list, enrichment analysis on gene ontology and pathway database presented upregulated process related to protein folding, heat shock proteins (HSPs) and response to ROS, representing direct protective mechanisms to HS. Other highlighted altered functions include extracellular matrix remodeling, immune response, and nutrients metabolism which might suggest cellular dysregulation or adaptation. Further study is required to confirm these effects. In conclusion, immortalized Hanwoo reproductive cells and molecular data in this study are valuable for further investigation of HS effect in cattle reproduction and developing preventive strategies.

Key words: *Bos taurus*, Hanwoo, cumulus, oviduct epithelial cells, endometrium epithelial cells, heat stress, livestock reproduction

Milk-derived extracellular vesicles enhance oocyte competence and embryo development during bovine *in vitro* maturation

Hyeong-Tae Jeon¹, Gyeong-Min Gim^{2,3}, Goo Jang^{3,*}, Junkoo Yi^{1,*}

¹School of Animal Life Convergence Science, Hankyong National University, Anseong 17579, Korea

²LARTBio Inc, Seoul 14322, Korea

³Department of Theriogenology, College of Veterinary Medicine and the Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Korea

Extracellular vesicles (EVs) are bioactive mediators increasingly applied to reproductive culture systems; however, the effect of milk-derived EVs on bovine oocyte maturation remains unclear. This study evaluated whether milk-derived EV supplementation during bovine *in vitro* maturation (IVM) improves oocyte competence. Cumulus–oocyte complexes were matured with milk-derived EVs at 1×10^7 , 1×10^8 , or 1×10^9 particles/mL, and oocyte maturation, embryo development, oxidative status, apoptosis/autophagy-related responses, and gene expression were assessed. EV-associated fluorescence signals were observed within cumulus–oocyte complexes after co-incubation with labeled milk-derived EVs. Milk-derived EV supplementation increased first polar body extrusion and cleavage rates, whereas blastocyst formation and hatching were most consistently improved in the 1×10^8 particles/mL group. This group also showed increased oocyte competence- and antioxidant defense-related gene expression, reduced H_2O_2 and NO levels, increased GSH levels, and decreased caspase-3 activity. In conclusion, milk-derived EV supplementation during bovine IVM improved oocyte maturation, at least in part, by reducing ROS-associated oxidative stress in oocytes.

Key words : milk-derived extracellular vesicles, bovine oocytes, *in vitro* maturation, oxidative stress, embryo development

Ultrasonographic ovarian morphological characteristics according to the interval from calving to conception in postpartum primiparous Hanwoo cows

Yonghwan Kim^{1,2}, Sung-Sik Kang¹

¹Hanwoo Research Center, National Institute of Animal Science, Rural Development Administration

²Department of Animal Resources Science, Kongju National University

Ovarian morphological parameters may serve as indicators of fertility in cattle. This study investigated whether ovarian morphological parameters at postpartum estrus are related to the interval from calving to conception in primiparous Hanwoo cows. Ultrasonography was performed on 16 primiparous cows at estrus and at 7 and 14 days after estrus to measure the diameter of ovary, follicle, and corpus luteum (CL), and intraovarian follicle and CL ratios. Artificial insemination was performed at each estrus, and conception was diagnosed at 30 days. Cows were classified as normal or abnormal estrus (Normal estrus: estrus interval ≥ 18 days, presence of CL at 14 days after estrus). Cows were also categorized into early (≤ 45 days, $n = 8$) and delayed conception groups (> 45 days, $n = 8$). Normal estrus showed greater ovarian diameter, CL diameter, and CL ratio at 7 days than abnormal estrus ($p < 0.05$). The early group had larger follicle diameter than the delayed group ($p < 0.05$). In the delayed group, CL diameter (1st vs. 2nd vs. 3rd estrus = 12.8 ± 2.7^a vs. 18.2 ± 1.4^b vs. 17.3 ± 1.5^b mm, $p < 0.05$) and CL ratio (1st vs. 2nd vs. 3rd estrus = 57.6 ± 5.5^a vs. 72.6 ± 3.5^b vs. $65.2 \pm 7.0\%^{ab}$, $p < 0.05$) at 14 days during the first postpartum estrus were lower than in subsequent cycles ($p < 0.05$). These results suggest that follicle diameter and CL development during the first postpartum estrus may be related to the interval from calving to conception.

Key words : Hanwoo cows, ovarian morphology, primiparous, ultrasonography

Integrating systematic labeling with machine learning for non-invasive reproductive stage detection in dairy cattle via feed intake patterns

Wookyung Lee, Dong-Hyeon Kim, Jae-Woo Song, Seung-Hyun Lee, Seongwon Seo, Wang-Hee Lee

Department of Smart Agriculture Systems Machinery Engineering, Chungnam National University,
Daejeon 34134, Korea

Reproduction is arguably the most foundational biological process in livestock production systems, as it directly governs the efficiency, profitability, and long-term sustainability of any operation. However, traditional methods for monitoring livestock reproductive cycles are often limited by high costs, labor intensity, and animal stress. While automated feeding systems provide continuous data, the link between raw feed intake and physiological reproductive status is not yet fully utilized. Leveraging the fundamental relationship between feeding behavior and reproductive cycles, this study investigates the feasibility of predicting specific reproductive stages exclusively through feed intake patterns. By integrating a systematic labeling strategy with machine learning, we defined six distinct gestational phases and developed a Random Forest (RF) model. The model classification accuracy averaged 0.916, and the F1-score was 0.905. Utilizing time-series feed intake features from dairy cows, this model effectively classifies reproductive stages based solely on feeding dynamics. The results demonstrated that feed intake patterns harbor inherent biological signals directly related to reproductive physiology. This research offers a significant technical contribution by establishing a non-invasive, cost-effective biomarker for reproductive monitoring. The proposed framework serves as a practical decision-support tool, facilitating precision reproduction management within the smart farming ecosystem.

Key words : precision reproduction, feed intake pattern, random forest, non-invasive monitoring, dairy cattle

Effects of canine-assisted interventions on neurophysiological and psychological responses

Youngwook Jung¹, Gaeun Jeong¹, Yujin Song¹, Kayoung Yang², Kyongwon Yoo³,
Youngtae Heo³, Minjung Yoon^{1,4,5,*}

¹Department of Animal Science and Biotechnology, Kyungpook National University, Sangju 37224, Korea

²Animal Welfare Division, National Institute of Animal Science, Wanju 55365, Korea

³Department of Companion Animal, Osan University, Osan 18119, Korea

⁴Department of Horse, Companion and Wild Animal Science, Kyungpook National University, Sangju 37224, Korea

⁵Research Institute for Innovative Animal Science, Kyungpook National University, Sangju 37224, Korea

Canine-assisted interventions (CAIs) have emerged as a promising complementary approach for improving mental health. Nevertheless, studies integrating both physiological and psychological measures to evaluate CAI effects remain limited. This study aimed to evaluate the neurophysiological and psychological effects of CAIs in humans under experimentally induced stress and to assess physiological responses in dogs. Sixteen healthy adults (8 males and 8 females) and three dogs (one male and two females) participated in a randomized crossover design. The Trier Social Stress Task (TSST) was administered prior to each condition to induce stress. Four conditions were tested: meditation, cognitive stress task, static activity, and dynamic activity. Meditation and the cognitive stress task served as control conditions. Static activity involved interaction with dogs, such as petting and talking to them. Dynamic activity consisted of higher-intensity, play-based interactions, such as agility and disc dog. In humans, salivary cortisol, electroencephalography (EEG), heart rate variability (HRV), and emotional states were measured at multiple time points before and after each activity. In dogs, salivary cortisol was measured repeatedly during each condition. Data were analyzed using linear mixed-effects models. Following static activity, concentrations of cortisol at the post-activity were lower than those observed after the cognitive stress task. In EEG, high-beta power decreased after the static activity. In HRV, SDNN and RMSSD increased following static activity. Following dynamic activity, high-beta power decreased in EEG, and the LF/HF ratio decreased in HRV. Physiological responses often varied by sex. Both static and dynamic activities improved emotional states. In dogs, concentrations of cortisol decreased after dynamic activity. Overall, these findings support beneficial physiological effects of CAIs in both humans and dogs, with additional psychological benefits observed in humans. These results highlight the importance of integrating multimodal measures when evaluating CAI outcomes.

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Key words: canine-assisted intervention, human-animal interaction, stress response, cortisol, electroencephalography, heart rate variability, emotional state

The effects of equine-assisted intervention on young adult women with depression

Gaeun Jeong¹, Suhyun Kim¹, Yujin Song¹, Youngwook Jung¹, Jae-Sung Lee²,
Hong-Gu Lee^{2,*}, Minjung Yoon^{1,3,4,*}

¹Department of Animal Science and Biotechnology, Kyungpook National University, Sangju, Korea

²Department of Animal Science, Konkuk University, Seoul 05029, Korea

³Department of Horse, Companion and Wild Animal Science, Kyungpook National University, Sangju, Korea

⁴Research Institute for Innovative Animal Science, Kyungpook National University, Sangju, Korea

Equine-assisted intervention (EAI) has gained attention as an alternative approach for improving emotional regulation and reducing stress in individuals with depression. This study investigated the neurophysiological and autonomic effects of EAI in young adult women according to depression status. Twenty female college students were classified into a depression group ($n = 10$) and a non-depression group ($n = 10$) based on Korean Beck Depression Inventory scores. The intervention consisted of 15 min of pre-counseling followed by 50 min of natural horsemanship-based ground activities. Electroencephalography (EEG) and electrocardiography (ECG) were recorded for 3 min before and after the program using QEEG-64FX. Relative power was assessed for the alpha (8–12 Hz), low-beta (13–19 Hz) and high-beta (20–30 Hz) frequency bands. All data were analyzed using PROC MIXED. EEG analysis showed a significant increase in alpha power after the intervention ($p < 0.05$), whereas low and high beta power showed no significant changes in either group. These findings suggest that EAI may promote relaxation and neural stabilization without inducing excessive cortical arousal. RR intervals extracted from ECG data were analyzed using Kubios software to calculate heart rate variability (HRV) indices. The PNS index significantly increased after EAI in both groups ($p < 0.05$), whereas the SNS index remained unchanged. RMSSD increased only in the depression group ($p < 0.05$), while SDNN and LF/HF ratio showed no significant changes. HRV results suggest that EAI may enhance parasympathetic regulation, particularly in young adult women with depressive symptoms, without inducing excessive physiological arousal. In summary, EAI may contribute to relaxation, neural stabilization, and autonomic stabilization in both depression and non-depression groups.

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Key words : equine-assisted intervention, depression, electroencephalography, heart rate variability, autonomic regulation