Doctoral Thesis

Studies on Roles of Inflammatory Mediators in the Mucosal Immune and Reproductive Functions in the Oviduct of Laying Hens

(Summary)

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March 2019

学 位 論 文 の 要 旨

論文題目　Studies on Roles of Inflammatory Mediators in the Mucosal Immune and Reproductive Functions in the Oviduct of Laying Hens

（産卵鶏卵管の粘膜免疫と生殖機能における炎症性メディエーターの役割に関する研究）

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Eggshell is a biophysical barrier against pathogens, its quality is associated with hatchability and production of healthy chicks. Eggshell quality requires healthy functions in oviductal mucosa, specifically the isthmus and uterus. Maintenance of mucosal homeostasis results from a complex interaction of several factors including the inflammatory mediators. Infection by pathogenic agents and aging of birds are assumed to modulate inflammatory mediators and affect the eggshell quality. However little information is available about their physiological functions in the chicken reproductive system. Thus, this study aimed to define the possible physiological roles of inflammatory mediators (prostaglandins (PG), cytokines, and chemokines) in regulating immune and reproductive functions in the oviduct of laying hens. Specifically, it was studied whether the infectious bronchitis virus (IBV)-antigen and aging modulated their expression in the oviduct. The expression of PG-producing enzymes (cyclooxygenase), cytokines, and chemokines during an ovulatory cycle was examined in the oviduct, including the isthmus and uterus for their importance in eggshell formation. Also, the effect of IBV antigen and aging on their expression and the impact on mucosal functions, namely mucosal innate immunity and eggshell formation were examined.

1. Expression and localization of cyclooxygenases in the oviduct of laying hens during the ovulatory cycle

Prostaglandins (PGs) are important regulators of functions of the hen oviduct. However, little is known about the rate-limiting cyclooxygenases (COX-1 and COX-2) in the oviduct. The aim of this study was to determine the COXs expression and localization in the different segments of the oviduct during an ovulatory cycle. White Leghorn laying hens were euthanized at 0, 4, 7, 16 and 24 h after oviposition, and samples from the infundibulum, magnum, isthmus, uterus, and vagina were collected. Gene and protein expressions of both COX-1 and COX-2 were examined by real-time PCR and western blot, respectively. Localization of COX-1 and COX-2 in the hen oviduct was determined by immunohistochemistry and PCR analysis of samples collected by laser capture microdissection. The expression level of COX-1 was highest in the infundibulum, while that of COX-2 was highest in the uterus. The expression levels of COX-1 in the infundibulum and COX-2 in the uterus were higher at 0 and 24 h after oviposition. Western blot analysis confirmed the presence of COX-1 and COX-2 in all oviductal segments. The density of COX-2 was highest in the uterus and did not change during the ovulatory cycle. COX-1 and COX-2 were localized in the surface epithelium of all oviductal segments besides the uterine tubular glands. Thus, it is

suggested that both COXs are expressed in all oviductal segments. COX-1 and COX-2 may play important roles in the infundibulum and uterus, respectively.

2. Effects of the infectious bronchitis virus antigen and prostaglandin E2 on the mucosal innate immunity in laying hens

Infectious bronchitis virus (IBV) causes deformities in eggshells. The aim of this study was to investigate the innate immune response to IBV, and whether prostaglandin (PG) E2 is involved in the innate immune response in the uterine mucosa. After treatment with IBV, the expression of viral RNA recognition receptors and innate antiviral factors were examined by real-time PCR and immunohistochemistry, and on PGE2 levels by ELISA. Then, the effects of PGE2 on the expression of innate antiviral factors in cultured uterine mucosal cells were examined. The results showed that the expression of RNA virus pattern recognition receptors (TLR3, 7, and MDA5), antimicrobial peptides (avian ß-defensins, including AvBD1, 2, 4-6; cathelicidins, including CATH1 and 3), and interferons (IFNα, ß, γ, λ), cyclooxygenase 2 (PG synthase) and the level of PGE2, the number of cells containing immunoreactive AvBD2 were increased by aIBV inoculation. In cultured mucosal cells, the expression of AvBD4, 10–13 and IFNα, ß, and λ was upregulated by incubation with 500 nM PGE2. These results suggest that IBV antigen increased the expression of innate antiviral factors, number of AvBD2 +ve leukocytes, and concentration of PGE2 in the uterine mucosa. PGE2 may support the uterine mucosal antiviral immunity by upregulating the expression of IFNs and AvBDs in the mucosal epithelial cells. Then, those antiviral factors may play roles in the protection against IBV.

3. Expression of pro- and anti-inflammatory cytokines and chemokines during the ovulatory cycle and effects of aging on their expression in the uterine mucosa of laying hens

The aim of the study in Chapter 4 was to examine whether cytokines and chemokines expressed in the uterine mucosa play a role in the process of eggshell formation in the chicken uterus. Thus, they were studied during an ovulatory cycle (experiment 1), and effects of aging on their expression (experiment 2) was examined. In experiment 1, the expression of the pro-inflammatory cytokines IL1ß, IL6, TNFSF15, and IFNγ, and a chemokine CX3CL1 was found to increase at 16 h following oviposition, while anti-inflammatory TGFß2 expression was found to increase at 4 h following oviposition. In experiment 2, a higher expression of the anti-inflammatory cytokines TGFß2 and TGFß3, and chemokines CXCLi2 and CX3CL1, was observed in aged hens than in young hens. A higher number of macrophages and CD8+ T cells were observed in the uterine tissue of aged hens than in young hens. This study concluded that the eggshell formation process may be affected by the pro- and anti-inflammatory cytokines and chemokines. The balanced expressions of these molecules might be disrupted in aged hens leading to a decline of eggshell quality.

4. Expression of collagen X and calcification-related molecules and effects of aging on them in laying hens.

Understanding why aged hens produce inferior quality eggs may help to produce long-life layers. Thus, the aim of this study was to determine the physiological expression of collagen (COL) X and calcification-related molecules (CRM) in the physiological egg formation process, and how they were affected by aging in laying hens. In experiment 1, the expression of COLX in the isthmic and uterine mucosa, and CRMs expression in the uterine mucosa during an ovulatory cycle was examined. In experiment 2, the expression of COLX in the isthmus and CRMs genes during shell formation in the uterus were examined in young (35 weeks old) and aged (130 weeks old) hens. The results showed that COLX was detected in the isthmus. The gene expression of CRMs plasma membrane Ca2+ ATPase (PMCA1), solute carrier (SLC) 26A9 and calbindin (CALB) was the highest at 16 h following oviposition, while the carbonic anhydrase (CA) 2 expression was the

highest at 7 h following oviposition. The expression of CALB and CA2 proteins was lower in aged hens than in young hens. These results suggest that eggshell biomineralization may be affected in aged hens by decreasing calcium transportation efficiency.

5. Age-related modulation of the isthmic and uterine mucosal innate immune defense system in laying hens

The aim of this study was to determine whether aging affected the mucosal tight junction (TJ) proteins, the synthesis of antimicrobial peptides (AMPs; including avian β-defensins (AvBDs) and cathelicidins (CATHs)), and Toll-like receptors (TLRs) in the isthmus and uterus of laying hens. Young and aged laying hens (35 and 130 weeks old, respectively) were used. The expression of TJ proteins, AvBDs, CATHs was examined by real-time PCR and western blotting. The results showed that the mRNA expression of TJ proteins, namely zonula occludin 2 in the isthmus and occludin in the uterus, was higher in aged hens than in young hens. The expression of two AvBDs in the isthmus and four AvBDs in the uterus was higher in aged hens than in young hens. However, the content of AvBD 1 and 11 was not altered by aging. Expression of TLR was higher in aged hens than in young hens in the isthmus. It can be concluded the potential ability to express TJ proteins and AvBDs in the isthmic and uterine mucosae of aged hens is higher than in young hens.

6. Conclusion

Inflammatory mediators including PGE2, cytokines, and chemokines could be expressed in the oviduct during an ovulatory cycle where they played roles in the reproductive and mucosal immune functions. The expression of COX1 and 2 were related to the engulfment of ovulated ovum and oviposition process, respectively. A physiological inflammation was regulated by the pro- and anti-inflammatory cytokines during an ovulatory cycle. The inflammatory mediators' expression is modulated by IBV-induced inflammation and aging. Recognition of microbial pattern molecules by TLRs induces COX2 expression and PGE2 production. PGE2 may support the antiviral innate immune response by inducing the expression of AMPs and IFNs. The increased levels of PGE2 may cause the premature oviposition of shell-less eggs. Aging might affect the uterine mucosal functions by disrupting the balance between the pro- and anti-inflammatory cytokines and it may cause a lowered production CALB and CA2 leading to a lower biomineralization efficiency. The recruitment of leukocytes into the uterine tissues of aged hens may be due to the increased expression of adhesion molecules or chemokines.

It is concluded that the inflammatory mediators are expressed strictly during an ovulatory cycle to regulate the reproductive and immune functions in the oviduct of laying hens, while disruption of their expression by IBV-induced inflammation or aging may affect these functions.