Form No.5

Abstract of Dissertation

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Title : Control mechanism of bovine oviduct function by the immune system
(ウシ卵管機能の免疫システムによる調節機構)

Abstract

The mammalian oviduct provides an optimal microenvironment for the activation and transportation of gametes, sperm capacitation, fertilization, and early embryonic development, which is critical for the establishment of a successful pregnancy. The oviduct is classically described as a sterile milieu even though pathogens and endotoxins can invade the mucosal surfaces of the oviduct via the uterus, peritoneal cavity, and follicular fluid. In addition, Sperm and the new embryo, which are allogeneic and semi-allogeneic agents, respectively, directly contact the oviduct epithelial cells, that could induce the immune responses in female. Therefore, the oviduct should be equipped with an efficient and strictly controlled immune system that would maintain optimal conditions for fertilization and early embryo development while provides protection against pathogens. In this study I aimed to reveal the oviductal immune responses to the different stimuli, pathophysiology vs. physiology.

In chapter 2, first, I investigated the regional distribution of immune cells in the bovine

oviduct *in vivo*. Histological studies revealed more abundant eosinophils (EOS) in the infundibula of the oviducts ipsilateral to the preovulatory dominant follicle and the ovulated ovary. The number of EOS was higher in the infundibula of the oviducts ipsilateral to the ovulated ovary than those of the oviducts contralateral to the ovulated ovary. The infundibula of the oviducts ipsilateral to the preovulatory dominant follicle had higher number of EOS than those of the oviducts ipsilateral to the mid-cycle corpus luteum. The number of EOS in the isthmus was higher in the outer layers (*tunica muscularis* and *tunica serosa*) than in the inner layers (*tunica mucosa* and *tunica submucosa*) during the estrous cycle. Thus, the EOS number varied with the region of the bovine oviduct, with greater number in the infundibula of the oviduct ipsilateral to the ovulated ovary, suggesting the impact of ovulation.

In chapter 3, I tried to reveal immunological responses of oviduct epithelial cells to the different stimuli. I studied the effect of *Escherichia coli* lipopolysaccharide (LPS) and its interaction with ovarian steroids, estradiol (E2) and progesterone (P4), and luteinizing hormone (LH) at concentrations observed during preovulatory period on immune responses in BOEC culture. Immunohistochemistry of oviduct tissue showed intensive expression of Toll-like receptor-4 (TLR-4) and TLR-2 in epithelial cells. A dose of 10 ng/ml LPS stimulated *TLR-4*, cyclooxygenase-2 (*COX-2*), nuclear factor-kappa B inhibitor A (*NFKBIA*), interleukin 1β (*IL-1β*), and tumor necrosis factor α (*TNF-α*) expression, indicating an early pro-inflammatory response. A dose of 100 ng/ml LPS did not induce expression of these genes but stimulated *TLR-2*, *IL-10, IL-4*, and microsomal prostaglandin E synthase-1 (m*PGES-1*) expression and PGE2 secretion, indicating an anti-inflammatory response. Ovarian steroids and LH completely block LPS (10 ng/ml)-induced *TLR-4*, *IL-1β*, and *TNF-α* expression as well as LPS (100 ng/ml)-induced *TLR-2* expression. These data suggest the existence of an early signaling system to infection in the BOEC. In addition, ovarian steroids and LH may play a critical role in inducing homeostasis and in controlling hyperactive pro-inflammatory responses detrimental to the epithelial cells, sperm and the embryo.

In chapter 4, I investigated function and regulation of an acute phase protein (APP), alpha-1-

acid glycoprotein (a-1-AGP) in the bovine oviduct and BOEC. AGP is one of the main APPs that are mainly produced by liver. Little is known about the local production and function of AGP. This study aimed to investigate the expression of AGP system *in vivo* and possible immune function of AGP in the BOEC *in vitro*. The results showed that the bovine oviduct expresses gene and protein for AGP, with the highest expression during postovulatory phase. Progesterone (P4 1 ng/ml) stimulated the expression of *AGP* and AGP receptor (*AGPR*). LPS (10 ng/ml, but not 100 ng/ml) stimulated the expression of *AGP* and *AGPR*. Sex hormones, E2, P4 and LH at concentrations observed during preovulatory period, suppressed LPS-induced *AGP* and *AGPR* expression. AGP (1-100 ng/ml) induced the expression of *TLR4* and *IL1β*, indicating a proinflammatory role for AGP. While, AGP (1-1000 ng/ml) suppressed the expression of *TLR2* and *TNFα*, an anti-inflammatory function for AGP. The co-stimulation of BOEC with LPS (10-100 ng/ml) and AGP (10-100 ng/ml) did not influence the LPS-induced *TLR4* and *IL1β* expression but inhibited LPS-induced *TLR2* and *TNFα* expression. To my knowledge, this is the first evidence showing the expression of AGP system in the bovine oviduct, indicating that AGP could be considered as a part of regulatory responses with the aim to silence some acute pro-inflammatory genes, and to maintain the possible expression of certain genes involved in the anti-infectious process.

In chapter 5, after investigation of immune response to LPS in the BOEC, pathophysiology, to reveal indirect response of oviduct immune system to the existence of a new embryo, BOEC was stimulated with interferon-tau which is a type I IFN, synthesized and secreted by the trophectoderm of the blastocysts. IFN-tau is regarded as the initial fetal signal required for maternal recognition of pregnancy in ruminant species. Recently, it has been shown that interferon-tau mRNA and protein are expressed in embryos on day 4 and on day 7 respectively. Therefore, I aimed to investigate the possible effect of very small amount of IFN-tau on the Th1/Th2 balance in the BOEC. IFN-tau at 1, 10, 100 and 1000 pg/ml dose-dependently induced the expression of TLR2, ISG15, TGFβ and IL1β; While, IFN-tau could not induce TLR4 and IL12. The results suggest that possibly very small amount of IFN-tau in the oviduct starts the change in the immune response toward Th2 response

which is related to a successful pregnancy.

Taken together, bovine oviduct epithelial cells respond to various stimuli differentially and act to keep one appropriate and constant condition that is necessary for a successful fertilization. This project suggests that bovine oviduct epithelial cells act to protect against pathogens while regulating the strength of proinflammatory responses that is harmful for cells. In this way, bovine oviduct epithelial cells employ ovarian steroids and LH to preclude over-expression of proinflammatory cytokines and Th1 response. These hormones as well as IFN-tau also favor a Th2 response, showing a critical role in maintaining an optimal Th1/Th2 balance in the oviduct. This study shows that oviductal epithelial cells via sex hormones act to stabilize the local immune status toward a Th2 response to support reproductive process like sperm capacitation, fertilization, embryo development and zygotes transportation. Therefore, the endocrine and autocrine systems in the oviduct influence reproductive health in dairy cattle as well as cow's soundness, and eventually result in higher cattle production.