Form No.5

Abstract of Thesis/Dissertation

Applicant

Doctoral Program of Animal Science and Agriculture

Graduate School of Animal and Veterinary Sciences and Agriculture

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 20380003

Name of Applicant: MANSOURI Alireza

Signature of Applicant:

Title : Computational modelling for molecular dynamics of TLR2 that regulates sperm-uterine immune crosstalk in cattle

(ウシの精子-子宮免疫クロストークを調節する TLR2 の分子動力学のコンピューターモデリング)

Abstract

In cattle, after artificial insemination (AI) or natural mating, a large number of sperm swim up into the female reproductive tract (FRT) toward the site of fertilization. During this journey, sperm interact with different compartments of the immune system of FRT. Bovine uterus has a well-regulated immune response to remove bacterial contamination after parturition, and it tolerates the allogenic sperm and accepts semi-allogenic embryos. Sperm generate transient proinflammatory response in the uterus which is required for the removal of dead/excess sperm with associated contaminants. TLR2 plays a central role in sperm-induced inflammation in the bovine uterus. In general, in immune cells, a dimerization of TLR2 with either TLR1 or TLR6 is required to activate intracellular signaling pathways, thereby inducing the innate immune response, but nothing is known about TLR2 dimerization in the bovine endometrial epithelium in response to sperm attachment. On the other hand, CD44 is a major cell surface receptor for hyaluronan (HA) involved in sperm attachment to the endometrial epithelium. In this study, using multiple approaches based on the computational modelling methods together with the *in vitro* experimental models, I was able to identify the major part of the molecular mechanism of sperm interaction with the bovine uterine immune system in cattle.

In chapter I, in order to test different TLR2 dimerization pathways in endometrium in an *in-vitro* model, 100 ng/mL TLR2 agonists (PAM3 as the TLR2/1 agonist, and PAM2 as the TLR2/6 agonist) were used to stimulate bovine endometrial epithelial cells (BEECs). Simultaneously, the expression of TLR1, 2 and 6 protein and gene in BEECs were investigated after exposure to sperm (5 million/mL). Further, sperm induced-inflammation was compared to PAM3 and PAM2 using the uterine explant *ex-vivo* model. The obtained data indicated that an activation of TLR2/1 signaling pathway in BEECs is involved in a weaker inflammation compared to TLR2/6. Moreover, similar to PAM3, sperm was able to induce TLR2 expression alongside with TLR1 in the uterus (gene and

protein), particularly in uterine glands, but not TLR6. In the same way, PAM3 and sperm could induce similar and low gene expression of pro-inflammatory cytokines (TNFA, IL1B and IL8) and TNFA protein to a lesser extent than PAM2 in the bovine endometrium. Thus, it is highly possible that sperm trigger endometrial epithelia to induce a weak inflammatory response through activating TLR2/TLR1 signaling cascade, which is needed to prepare an ideal environment for embryo reception. Afterwards, *in-silico* approaches were employed to investigate and confirm TLR2 dimerization in bovine species (TLR2/1 or TLR2/6). Homology modeling methods were used to determine the 3D protein structure of bovine TLRs. The *in-silico* findings suggested that the stability of TLR2 dimerization is heavily depending on the presence of the bridging agonist in bovine, which is similar to human and mouse species.

In chapter II, I hypothesized that HA may act as a bridging ligand between sperm and CD44/TLR2 of BEECs. To test the above hypothesis, I first determined the binding affinity of HA to CD44 and TLR2 molecules. my *in-silico* model revealed that low molecular weight HA molecules have a higher affinity to CD44- than TLR2 interaction. Next, HA existence in bovine endometrium was investigated via immunostaining using a biotinylated HA-binding protein. Notably, HA is localized in the luminal and glandular endometrial epithelia. Moreover, ELISA showed detectable levels of HA (16.05 ± 2.33 ng/ml) in BEECs-conditioned medium. As a result, BEECs were treated with different concentrations of low molecular weight HA (at 0, 0.1, 1, or 10 µg/mL) for 2 h prior to the co-culture with 10^6 sperm/mL for additional 3 h. Importantly, HA dose-dependently increased the number of sperm attached to BEECs. Besides, the quantitative real-time PCR data illustrated that supplementation of BEECs with HA (at 1 µg/mL) upregulate mRNA expressions of TLR2, pro-inflammatory- cytokines (TNFA and IL1B) and chemokines (IL8) as well as prostaglandins E synthesis (PGES) in BEECs in response to sperm. However, BEECs treatment with HA only (no sperm exposure) did not show any significant difference in transcriptional levels of the selected genes when compared to the non-treated BEECs. Collectively, the findings provide evidence that HA, primarily through CD44 interaction, has the capacity to facilitate sperm attachment to the endometrial epithelia with a subsequent TLR2-mediated immune response.

Overall, my findings in chapter I revealed that sperm activate TLR2/1 heterodimerization, but not TLR2/6, to trigger a weak physiological inflammatory response in bovine endometrium. The data of chapter II suggested that sperm keep a higher affinity for attaching to the BEECs in presence of HA through interaction with CD44, consequently inducing proinflammatory response through TLR2 signaling pathway. Collectively, this weak inflammation triggered by sperm with the molecular network of TLR2/1, CD44 and hyaluronan must be the specific way to remove excess/dead sperm remaining in the bovine uterine lumen without tissue damage for providing the ideal environment for embryo implantation.