Form No.5

Abstract of Dissertation

Applicant

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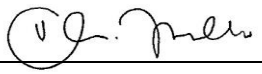
Signature of Applicant: 

Title：Dynamics of sperm-oviduct interaction that regulates maternal immune response in cattle

（ウシ母体の免疫応答を制御する精子と卵管の相互作用ダイナミクス）

Abstract

The oviduct is an active and dynamic organ that supports functions critical for reproduction, such as sperm storage, sperm capacitation, fertilization, and early embryonic development. Once sperm reach the oviduct, many attaches to the epithelium that lines the caudal isthmus, forming a sperm reservoir. In the peri-ovulatory period, sperm move up to the ampulla of the oviduct, where fertilization and the first few days of embryonic development occur. While much has been learned about sperm interaction with the isthmus, very little is known about the interaction of sperm with the ampulla, despite its role in fertilization.

In chapter 1, a differentiated explant model using bovine oviductal ampulla obtained from the pre-ovulatory phase was developed to study the interaction between the sperm and the oviductal epithelium. The explants were derived from the primary fold of the oviductal ampulla and is approximately 3mm x 3mm in size. The explant was incubated in PEG-supplemented DMEM under a 38.5˚C incubator with 5% CO_2 in humidified air and was subjected to microscopic observation of morphology under phase contrast and fluorescence microscopy. Hematoxylin and eosin-preparations of the explant was also used for further observation of the complex tissue architecture. The explant is comprised of ciliated and non-ciliated cells. The motile cilia are beating synchronously which caused the explant to move around the media. The explant was incubated with sperm to observe the interaction. Mitochondrial stain JC1 was used to stain the midpiece of sperm for illumination. The sperm attached immediately to ciliated cells with the sperm head bound tightly by the cilia. The sperm bound uniformly all over the explant surface and are tangentially attached to the epithelium. The beating of the ciliated epithelial cells allowed the sperm to orient in similar direction. Viability evaluation showed that sperm and

explant optimal condition for incubation is maintained up to 6 h. The results show that the explant model is a suitable model for observation of the interaction between the sperm and the intact oviductal ampullary tissue.

In chapter 2, a detailed study on sperm-oviductal ampulla interaction and its impact on sperm attachment and immune response of the oviductal tissue was carried out using the differentiated explant model comprised of the pre-ovulatory phase bovine oviductal ampulla. Sperm capacitation was induced by incubating the sperm with heparin while non-treated sperm served as the control. Both heparin-treated and non-treated sperm attached in equal numbers to the explant. Despite no detectable difference in the sperm attachment, only the heparin treated sperm stimulated mRNA transcription of *TLR2, TGFB1, PGES,* and *IL8,* whereas *TNFA and IL10* were not affected. In the non-treated sperm, only *PGES* was upregulated. The results show that the explant epithelium is more immunologically sensitive to the sperm treated with heparin for capacitation. Somehow, heparin allowed modifications on the sperm membrane which facilitated the interaction with the TLR2.

In chapter 3, the explant model was used to study the involvement of the TLR2 in the sperm-oviduct ampullary explant interaction and immune response of the explant to the presence of the sperm. The TLR2 was blocked using a specific TLR1/2 antagonist. Afterwards, the sperm attachment to explant, gene transcription, and TLR2 protein expression and localization in the explant were evaluated. Adding the TLR1/2 antagonist in the coincubation system for either types of sperm resulted in a reduction of the attachment at 5 min and 15 mins. The TLR1/2 antagonist also effectively blocked the sperm-induced mRNA expression for *TLR2, TGFB1,* and *IL8.* The attachment of the sperm to the explant also resulted in an intense TLR2 protein expression of the oviductal ampullary epithelium. However, the tissue response to sperm was ablated by the addition of the TLR1/2 antagonist into the coincubation system.

It can be concluded that the bovine differentiated explant model developed in this study can be used to observe the sperm interaction with the intact and highly complex oviductal tissue. The model provided detailed observations on the dynamics between the sperm and intact ampullary epithelium. Although both heparin-treated and non-treated sperm showed the same affinity for the explant epithelial cells, only the sperm incubated with heparin stimulated an anti-inflammatory immune response in the explant which may serve to protect the sperm in the oviduct. The response is accompanied by increased transcription and translation for the TLR2 protein. Blocking the TLR2 gives the evidence that the TLR2 mediates the immune response of the pre-ovulatory ampullary epithelium to sperm binding. Further studies are needed to elucidate the detailed mechanism of the sperm-oviduct interactions and the identification of the ligands involved in the interaction and subsequent immune response.