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Abstract of Dissertation

Applicant

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Title：Disruption of early embryo-maternal crosstalk *via* peptidoglycan in the infected uterus in the cow

(細菌感染したウシ子宮内におけるペプチドグリカンによる初期胚ー母体のクロストーク阻害)

Abstract

Bidirectional embryo-maternal communication is critically important for appropriate maternal recognition of pregnancy and thereby establishing a successful gestation. Any alteration in maternal recognition of pregnancy may result in early embryonic loss. Bio-molecular dialogue between the embryo and mother should be started by developing bovine embryo in order to establish a suitable uterine environment that could support the growth of developing embryo leading to successful pregnancy. In case of ruminant, interferon tau (IFNT) is secreted from the trophoectoderm and acts as a signaling molecule for recognition of pregnancy.

After parturition, natural orifice such as vaginal opening remains open. Soon after parturition, bacteria present in feces, urine, and bedding materials can enter into the uterus and may cause infection. Moreover, biodegradable materials present within the uterus after partition serve as a good media for the growth of a variety of aerobic and anaerobic bacteria. These perceptions become more complicated with the fact that the gravid uterus is not germ-free as it has been thought for many years, and it may be colonized with numerous bacteria including *Trueperella pyogenes*. These bacteria persistently remain within the uterus and continue to grow. As a result, peptidoglycan (PGN), the important cell component of bacteria is continuously producing as byproduct which remains within the uterine environment. It has been reported that PGN administered after 5 days of mating interrupts early pregnancy in ewes *in vivo*. Therefore, I hypothesized that presence of PGN within the uterine environment has deleterious effects on embryo-derived IFNT mediated embryo-maternal immune crosstalk.

In chapter 1, I used embryo conditioned media (ECM) to see the effect of embryo culture media on bovine endometrium epithelial cells (BEECs) in presence or absence of PGN. I used this model to

mimic *in vivo* real situation where the uterus contains persistent bacterial infection and the embryo is coming and/or transferring to that uterus. Healthy bovine uteri of luteal phage (D 7-8) were collected from local slaughterhouse for isolation and culture of epithelial cells. When the cells from second passage reached at 80-90% confluence, then cells were cultured in ECM in presence or absence of PGN for 24 h in a 38.5^{0}C incubator with 5% CO_2 in humidified air. Cells without ECM or PGN served as control. BEECs were analyzed to see the *mRNA* expressions of *ISGs* (*OAS1* and *ISG15*) and *STAT1*, a key factor for IFNT signaling by real-time PCR. Real-time PCR result demonstrated that ECM induced *mRNA* expression of *ISGs* (*OAS1* and *ISG15*) and *STAT1*, a crucial molecule for IFNT signaling. On the other hand, presence of PGN significantly suppressed the upregulation of *ISGs* (*OAS1* and *ISG15*) and *STAT1* in BEECs. Surprisingly, no modulatory effects on the *mRNA* expression of pro-inflammatory cytokine (*TNFA* and *IL1B*) or *TLR2* were observed by PGN. The findings strongly suggest that ECM contains IFNT secreted from cultured embryos, and that PGN blocks IFNT signaling in BEEC.

In chapter 2, I investigated the direct effect of IFNT, secreted from embryos, on BEECs *in vitro* and endometrial explants *ex-vivo* in presence or absence of PGN. Similar to chapter 1, when the cells from second passage reached 80-90% confluence, cells were stimulated with IFNT in the presence or absence of PGN for 24 h. Healthy uteri of the same stage were obtained and using an 8 mm biopsy punch, disks of endometrial tissue (2 mm thick) were separated from the intercaruncular regions ipsilateral *to* the corpus luteum. Then explants were cultivated in Roswell Park Memorial Institute (RPMI)-1640 media supplemented with 10% fetal bovine serum (FBS) with IFNT in the presence or absence of PGN for 12 h in a 38.5^{0}C incubator with 5% CO_2 in humidified air. Cells/explants without IFNT and PGN served as control. BEECs and endometrial tissues were analyzed for *mRNA* expressions of *ISGs* (*OAS1* and *ISG15*) and *STAT1*, a key factor for IFNT signaling, by real-time PCR. OAS1 protein was identified by immunofluorescence in LE of endometrial explants. Gene analysis results showed that IFNT stimulated the expression of interferon stimulated genes (*ISGs: OAS1, ISG15*) and *STAT1* in BEECs *in vitro* and explants *ex-vivo*. While the presence of PGN significantly suppressed the upregulation of *ISGs* and *STAT1* in both models. Interestingly, PGN did not stimulate *mRNA* expressions of the pro-inflammatory cytokines (*TNFA* and *IL1B*) or *TLR2* in BEECs or explants. I also analyzed *interferon receptor 1 (IFNAR1)* and *IFNAR2* to see the any fold expression changes in explant. Real-time-PCR result showed no changes of *mRNA* expression of *IFNAR1* and *IFNAR2* among all groups. Further, OAS1 protein expression was detected using immunofluorescence (IF) staining in the luminal epithelia of endometrial tissue, and PGN suppressed the IFNT-triggered intensive OAS1 protein expression throughout the whole luminal epithelium.

It can be concluded that the findings of this study demonstrate that the presence of low levels of PGN suppresses early embryo-derived IFNT signals for *ISGs* expression in the bovine endometrium and thereby, interferes with endometrial epithelium receptivity and early embryo-maternal crosstalk in cows, which leads to pregnancy failure.