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# Abstract of Thesis/Dissertation

Applicant

Master's/Doctoral Program in Animal and Food Hygiene

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Title : The local immune response to early embryo in bovine oviduct and uterus *in vitro*

(ウシ卵管と子宮の体外培養系における初期胚に対する局所免疫応答)

Abstract

The mucosal immune system of the female reproductive tract (FRT) plays dual roles by accepting allogenic spermatozoa and semi-allogenic embryo, whilst provides protection against pathogens. Following fertilization, the bovine embryo stays in the oviduct for about 4 days and enters into the uterus at approximately the 16-cell to morula stage. In the uterus, the embryo develops to blastocyst by D7 and hatches from the zona pellucida between D9 and D10 of pregnancy. Recent investigations suggest that the embryo-maternal communication starts in the bovine oviduct (D0-D4) and uterus immediately after arrival of morula until hatching of blastocyst (D5-D9). The early developing embryo expresses paternal antigen, and thus it could be considered as foreign to the bovine oviduct and uterus. However, the semi-allogenic embryo somehow escapes from attack by maternal immune system and establishes pregnancy. The molecular mechanism involved in acceptance of semi-allogenic embryo in the bovine oviduct and uterus, particularly at very early stage of pregnancy, remains mostly unknown. Interferon tau (IFNT) is an embryo-derived pregnancy recognition signal in cattle that inhibits the luteal regression and thereby maintains pregnancy. In addition, it has anti-proliferative and immunosuppressive properties. *IFNT* mRNA is expressed in 8-16 cell bovine embryo and its protein can be detected in morula and non-hatched blastocyst on D7. Therefore, it is possible that a small amount of IFNT regulates local immune response in the bovine

oviduct and uterus at very early stage of pregnancy in cattle. All together, the present study aimed to investigate the effect of early developing embryo on the immune-related gene expression in bovine oviduct epithelial cells (BOECs) and uterine epithelial cells (BUECs) *in vitro*. Further, the current study examined the effect of embryo-BOECs and embryo-BUECs interaction, or embryo alone *via* conditioned media (CM) on gene expression in peripheral blood mononuclear cells (PBMCs).

In chapter I, BOECs were co-cultured with zygotes (n=25-30) and without zygotes (control) for D0-D4 (D0=IVF), and gene expression in BOECs and developing embryos was analyzed. ELISA was performed to determine IFNT concentration in CM from embryo-BOEC co-culture. Subsequently, PBMCs were cultured in CM from embryo-BOEC co-culture or BOEC culture (control), and gene expression was evaluated. Next, zygotes (n=25-30) were cultured alone without BOECs for D0-D4. At the same time, fresh medium was also incubated for D0-D4. PBMCs were cultured in CM from embryo culture or in CM without embryos (control), and gene expression was evaluated. In BOECs, the developing embryos did not induce interferon (IFN)-stimulated genes (*ISGs*: *ISG15*, *OAS1* and *MX2*), a key factor for IFN-signaling (*STAT1*), type-1 IFN receptors (*IFNAR1* and *IFNAR2*), but stimulated *PTGES* (an enzyme for PGE2 synthesis) and suppressed *NFkB2* and *NFkBIA* (key factors for inflammatory and immune response). Interestingly, in PBMCs, CM from embryo-BOEC co-culture stimulated *ISGs*, *STAT1*, *PTGES* and *TGFB1* (Th2 cytokine), but suppressed *IL17* (Th17 cytokine). In contrast, CM from D0-D4 embryo culture alone did not influence *ISGs* and other immune-related genes expression in PBMCs. *IFNT* and *PTGES* were expressed in the 16-32 cell embryos developed on BOEC monolayer at the end of culture. It was not possible to determine IFNT concentration in CM of embryo-BOEC co-culture.

In chapter II, BUECs were co-cultured with morulae (n=10) and without morulae (control) for D5-D9 (D0=IVF), and gene expression in BUECs was analyzed. ELISA was performed to determine PGE2 and IFNT concentration in CM from embryo-BUEC co-culture. Further, PBMCs were cultured in CM from embryo-BUEC co-culture or BUEC culture (control), and gene expression was evaluated. Next, morulae (n=10) were cultured alone without BUECs for D5-D9. Simultaneously, fresh medium was also incubated for D5-D9. PBMCs were cultured in CM from embryo culture or in CM without embryos (control), and gene expression was analyzed. Finally, BUECs and PBMCs were treated with IFNT (100 pg/ml) for analysis of gene expression. In BUECs, the developing embryos

induced *ISGs*, *STAT1*, *IFNAR1* and *IFNAR2*, with suppression of *NFkB2*, *NFkBIA* and pro-inflammatory cytokines (*TNFA* and *IL1B*). The embryos also stimulated *PTGES* in BUECs. In accordance with gene expression, the developing embryos stimulated PGE2 secretion by 20-fold from BUECs with compared to control; however, it was not possible to determine IFNT concentration in CM of embryo-BUEC co-culture. In PBMCs, both CM from embryo-BUEC co-culture and embryo culture alone induced *ISGs*, *STAT1* and *TGFB1*, while suppressing *TNFA* and *IL17*. Similar to the embryo, IFNT at 100 pg/ml suppressed *NFkB2*, *TNFA* and *IL1B* in BUECs, and also stimulated *TGFB1* and suppressed *TNFA* in PBMCs.

Taken together, in the oviduct, the results suggest that the early cleavage-stage embryo starts to secrete IFNT in the bovine oviduct, which is recognized by the immune cells with a help of epithelial cells secretion. The developing embryo induces an anti-inflammatory response in the oviduct epithelial cells without stimulation of *ISGs*; however, the embryo alone cannot regulate gene expression of immune cells. An interaction between the developing embryo and BOECs modulates gene expression of immune cells (PBMCs) towards anti-inflammatory action with activation of *ISGs*. In the uterus, the findings suggest that the bovine embryo, in the first four days in the uterus (D5-D9), starts to induce an anti-inflammatory response in epithelial cells with activation of *ISGs*. In addition, the developing embryo during this period of development is capable of regulating gene expression of the immune cells towards suppression in the bovine uterus. A small amount of IFNT form the early developing embryo is likely to be involved in modulation of such "local" immune response in the bovine oviduct and uterus.

Finally, the present study has provided basic information for understanding the molecular crosstalk between the early developing embryos and the immune cells in the bovine oviduct and uterus. The findings of this study could be useful to make further plan for reducing the rejection of semi-allogenic embryo by the maternal immune system, and thereby improve the fertility and productivity of the cows. It should also be noted that the pathogenic infections, heat stress and any other stress inside the herd may disrupt the physiological immune response of the FRT to the developing embryo in cows. Thus, appropriate hygiene should be strictly maintained in the herd to maximize the fertility and productivity of the cows.