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Abstract of dissertation

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Title : The role of interferon-tau (IFNT) secreted from Day-7 embryo in uterine immune modulation in cattle

( ウシの7日目の初期胚から分泌されるインターフェロン・タウの子宮内免疫調節の役割 )

## Abstract

Immune modulation in the uterus at the embryo-maternal interface is critically important for establishing successful pregnancy in cattle. Any disruption of the immune response in the uterus may lead to early embryonic rejection. The early bovine embryo needs to start a bio-molecular dialogue with the maternal immune system to set up a modified immune response to the embryo. The unattached embryo suspending in the uterine fluid releases several bioactive signaling molecules that initiate dialogue with mother. Interferon-tau (IFNT), a trophectoderm derived embryonic signal molecule, is to be considered as one of the main factors in embryo-uterus crosstalk during early pregnancy. IFNT is a maternal recognition signal and maintains pregnancy by protecting corpus luteum from lysis. Apart

from its anti-luteolytic function, IFNT is being reported as an immune suppressive molecule to the immune cell. Moreover, local uterine immune cells, as well as circulatory immune cells, responded to conceptus-derived IFNT suggesting that there might be IFNT mediated immune regulation exists in the uterus. Till now, there is little or no information regarding direct evidence of IFNT involvement in immune modulation at the period just after the embryo moves from the oviduct to the uterus. However, the semi-allogenic bovine blastocyst avoids the maternal immune attack and establishes pregnancy in the uterus in cattle. Recent studies suggest that Day-7 bovine embryo starts to communicate with the uterine epithelium through IFNT signaling. Therefore, I hypothesized that Day-7 bovine embryo secretes very small amount of IFNT in the uterus which then communicates locally with the uterine epithelium for generation of an anti-inflammatory response in immune cells. To examine the hypothesis, the present study aimed to investigate the effect of uterine flush (UF) from Day-7 pregnant cow (*in vivo*) on interferon-stimulated genes (*ISGs)* as well as immune-related genes in peripheral blood mononuclear cells (PBMCs). Further, the current study examined whether or not IFNT directly regulated this immune-related gene expression in PBMCs through the use of bovine uterine epithelial cells (*in vitro*) and cow uterus (*in vivo*).

In Chapter I, in *in vivo* study, the effect of UF from Day-7 pregnant cow with multiple embryos on gene expressions in PBMCs was investigated. First, cows were superovulated by using routine hormonal treatment for superovulation regimen of embryo transfer program. After estrus (Day-0), cows were either inseminated (n=12; pregnant group) or remained non-inseminated (n=5; control group). On Day-7, the first UF was collected (20-25 ml) and pregnancy was confirmed with the presence of multiple embryos in UF. Subsequently, PBMCs were then cultured in UF of all groups and gene expressions were analyzed.

Transcripts detected in PBMCs revealed that UF from pregnant cows down-regulated pro-inflammatory cytokines (*TNFA, IL1B)* and up-regulated anti-inflammatory cytokine (*IL10)* expression, with activation of interferon-stimulated genes (*ISGs; ISG15, OAS1)* as compared with UF from non-pregnant cows. An addition of specific anti-IFNT antibody to the UF inhibited the effect on PBMCs, indicating that IFNT is a major factor for such immune modulation. In *in vitro* study, the effect of IFNT-stimulated BUEC-CM on immune-related gene expressions in PBMCs was analyzed. First, bovine uterine epithelial cells (BUECs) after first passage were stimulated with IFNT (100 pg/ml) for 24 h and conditioned media (CM) was collected (IFNT-stimulated CM). CM from BUECs without IFNT stimulation served as controls and CM supplemented with IFNT (100 pg/ml) served as recombinant IFNT-CM. Subsequently, PBMCs were cultured in IFNT-stimulated CM (*in vitro*, indirect) or CM supplemented with IFNT (*in vitro*, direct) and gene expressions were analyzed. The observation that conditioned media from BUEC both stimulated with IFNT *in vitro* and supplemented with fresh IFNT induced similar gene expression in PBMCs, confirming that IFNT directly acts on this immune crosstalk.

In Chapter II, the effect of IFNT-infused UF on immune-related gene expressions in PBMCs was investigated. First, cows (n=5) were synchronized to estrus and remained non-inseminated. On Day-6 fresh RPMI-1640 media (100 ml) was infused into the uterus, incubated for 24 h and UF was collected (served as control-1) on Day-7. A similar procedure was done again for infusion of fresh media on Day-7 and collection of UF (served as control-2) on Day-8. Immediately after collection of UF, an exogenous IFNT (100 pg/ml) with the same volume of RPMI-1640 media (100 ml) was infused into the uterus and UF was collected after 24 h incubation (served as IFNT-infused UF) on Day-9. Subsequently, PBMCs were

cultured in UF with or without IFNT infusion for 24 h and gene expressions were analyzed. Gene analysis revealed that UF infused with IFNT down-regulated pro-inflammatory cytokines (*TNFA, IL1B)* and up-regulated anti-inflammatory cytokine (*TGFB1)* and *PTGES* expression, with activation of interferon-stimulated genes (*ISGs; ISG15, OAS1)* as compared with UF infused with fresh media (control-2, Day-8). This result indicates that exogenous IFNT alone or co-action with other factors secreted from uterine epithelial cells can mediate local anti-inflammatory immune condition for immune cells.

Altogether, the results of the present study support the hypothesis that IFNT secreted from Day-7 bovine blastocyst modulates local uterine immune environment towards an anti-inflammatory response in immune cells, which could play a pivotal role in the immunological acceptance of the blastocyst in the uterus. Furthermore, exogenous IFNT instead of embryo-derived IFNT can also induce such immune regulation in the uterus during this period of pregnancy. Therefore, it is likely that IFNT at a low dose (100 pg/ml) along with embryo may help in driving the environment for tolerance of embryo in the uterus.

The present study has provided new insight into the molecular mechanism by which a semi-allogenic Day-7 blastocyst escapes from attack by the maternal immune system and is accepted in the uterus in cows. The findings of this study could be useful to make a further plan of IFNT (100 pg/ml) infusion in the uterus at the time of embryo transfer (ET) on Day-7, for assuring acceptable immune-environment in the uterus to accept the embryo. This could be one of the ways for achieving high reproductive performance in dairy cows.