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Pregnancy specific regulation of lysosomal cathepsins in bovine blood leukocytes

（ウシ末梢白血球におけるリソソームカテプシンの妊娠特異的応答機構に関する研究）

In ruminants, interferon tau (IFNT) is an important pregnancy recognition signal which is secreted from the trophoctoderm of the conceptus at the blastocyst stage. IFNT-mediated expression of interferon stimulated genes (ISGs) in peripheral blood leukocytes (PBLs) can indicate pregnancy. Recently, activation of lysosomes and lysosomal cathepsins (CTSs) by type 1 IFN signal transduction pathway was observed in immune cells. This study was conducted to explore the dynamics of lysosomes and lysosomal CTSs in bovine leukocytes during early pregnancy.

1. Pregnancy specific response of lysosomal CTSs in bovine leukocytes

This study aimed to explore the IFNT-mediated lysosomal activation in PBLs during early pregnancy of cows. Multiparous Holstein Friesian cows were subjected to artificial insemination (AI). Leukocytes collected from peripheral bloods on d18 of pregnant and non-pregnant cows were separated and used for the measurement of lysosomal acidification, activities of CTS B and K, expression of *LAMP-1*, -2 and CTS *B* and *K* genes. Lysosomal acidification, CTS B and K activities were significantly increased in the pregnant leukocytes than those collected from non-pregnant cows. Gene expression levels of *LAMP-1*, -2 and CTS *B* and *K* were also significantly increased in the pregnant leukocytes than non-pregnant cells. Besides, immunodetection showed significant increase of CTSK and LAMP-1 in the leukocytes of pregnant cows. These results showed the first observation of pregnancy-specific lysosomal activation in bovine PBLs.

2. Status of lysosomes and lysosomal CTSs in bovine PBMCs and PMNs during early pregnancy

This study aimed to investigate the cell specific difference of lysosomal activation in the separated peripheral blood mononuclear cells (PBMCs) and polymorphonuclear granulocytes (PMNs). Blood samples were collected on d0, 7, 14 and 18 after AI. After 40 days of AI, pregnancy was confirmed by ultrasonography. The activities of CTSs B, K and L were increased significantly both in PBMCs and PMNs in the progress of pregnancy. Expression levels of CTS *B* and *K* were significantly increased both in PBMCs or PMNs in pregnant cows compared to non-pregnant cows.

Assessment of lysosomal activity was also showed higher and increased significantly in PBMCs and PMNs in pregnant cows. Expression levels of *LAMP-1*, -2 were increased significantly in pregnant cows compared to non-pregnant cows on d18. Immunodetection analysis revealed that, CTSB protein was detected and significantly increased in PBMCs and PMNs in pregnant cows compared to non-pregnant cows on d18. The findings of this study suggest that, lysosomal CTSs could be reactive to IFNT in separated PBMCs and PMNs. It also indicated that PMNs were more sensitive than PBMCs and could be used as a potential marker cells for detection of early pregnancy.

3. Effect of IFNT on stimulation of the activity of lysosomes and lysosomal CTSs in bovine PBMCs and PMNs

To elucidate the direct effect of IFNT secreted from conceptus for the activation of lysosomal functions, *in vitro* experiment with recombinant IFNT (rbIFNT) was performed. Addition of IFNT significantly increased the activities of CTSs B, K and L both in PBMCs and PMNs. Gene expression levels of CTS *B* and *K* were also increased significantly both in PBMCs and PMNs by IFNT stimulation. Lysosomal activity was increased significantly in PBMCs and PMNs on d18. Immunodetection analysis revealed that CTSB protein was detected and significantly increased in PBMCs and PMNs. These results suggested that CTSs activity is increased by the direct IFNT stimulation in PBMCs and PMNs and supported the lysosomal function.

4. Involvement of type 1 IFN signal transduction pathway on lysosomal function in bovine leukocytes

The pregnancy-specific lysosomal activation of blood leukocytes is highly possible by IFNT. However, IFNT stimulation on PBL mechanism is unclear. Therefore, this experiment was conducted *in vitro* stimulation of non-AI blood leukocytes by IFNT treatment with specific inhibitor for JAK/STAT pathway. Lysosomal acidification and expression levels of *LAMP-1*, -2 as wells as activities of CTSB and K were significantly increased by IFNT. In contrast, addition of AZD1480, a JAK inhibitor, significantly decreased the lysosomal acidity and CTSK activity, expression of *LAMP-1*, -2 and *CTSK* in the presence of IFNT. Immunodetection also showed a significant increase of CTSK and LAMP-1 after IFNT stimulation and decreased significantly after JAK inhibitor treatment in the presence of IFNT. These results suggest that lysosomal function in leukocytes is regulated by IFNT via a type I IFN mediated pathway at the time of pregnancy recognition.

It is concluded that, the lysosomes and lysosomal CTSs activity, expressions and protein level could be responsive to IFNT during maternal-fetal recognition period of pregnancy in PBLs via type I IFN signal transduction pathway and also lysosome and lysosomal CTSs potentially be useful biomarkers for early pregnancy detection.