



# HOKKAIDO UNIVERSITY

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Effects of milk osteopontin on the normalization of endometrial epidermal growth factor profile and restoration of fertility in repeat breeder dairy cows  
(乳由来オステオポンチンが乳用リピートブリーダー牛における子宮内膜上皮成長因子発現の正常化と受胎性回復に及ぼす効果)

Endometrial EGF concentrations show a cyclic change with two peaks on days 2-4 and 13-14 during the estrous cycle. Loss of the two peaks has been linked to reduced fertility in repeat breeder cows. A form of osteopontin (OPN) found in bull seminal plasma (SP) normalized the EGF profile and restored fertility in repeat breeder cows. The aim of this study was to examine the effects of OPN from milk (mOPN) on the normalization of the endometrial EGF profile and restoration of fertility in repeat breeder cows. In Chapter 1, effects of mOPN to normalize the EGF profile and restore fertility were examined. OPN was purified from 1 L of milk. Three major protein bands were identified as OPN by Western blotting. Their tryptic peptide masses matched approximately 50, 40 and 10%, respectively, by peptide mass finger printing. Sum of the three detected bands accounted for approximately 85% of total protein contents of the OPN preparation. OPN was obtained between 6.3 and 23.4 mg from 1 L. Then, mOPN (1.3 mg), SP (0.5 mL), and PBS were infused into the vagina. The normalization rate of the EGF profile of mOPN infusion (56.1%) was similar to that of SP (58.1%) and higher than that of PBS (23.8%). The conception rate after the infusion of mOPN (43.5%) was similar to that of SP (40.0%) and higher than that of PBS (22.2%). The results demonstrated the effect of mOPN on the normalization of the EGF profile. However, using OPN from raw milk for the treatment of many other cows carries the risk of infections. In Chapter 2, therefore, I have examined the feasibility of treatment protocol for the repeat breeder cow with mOPN prepared from her own milk to avoid the risk of disease transmission through the treatment. OPN content was measured in milk samples from apparently normal and repeat breeder cows with different lactation stages. OPN contents were higher in the colostrum than the other stages. The contents of OPN in repeat breeder cows were similar to those of the control cows. OPN was obtained 1.0 to 3.3 mg from 100 mL of milk. Within control cows, the proportion of three bands differed

between the stages. When the proportion of the three bands were compared between the control and repeat breeder cows, the proportion of the bands differed by the type of cows but not by the lactation stages. After the infusion of mOPN (1 mg) prepared from the normal and repeat breeder cows, both OPN preparations normalized the EGF profile at similar rates (63.3% and 60.0%, respectively) and the rates were higher than that of the PBS (25.7%). However, effects of mOPN from repeat breeder cows own milk on fertility remained to be confirmed since the conception rate were at an intermediate level of PBS and mOPN from normal cows. The results indicated that mOPN from individual repeat breeder cows could be an effective treatment option to enabling normalization of the endometrial EGF profile and avoiding the risk of spreading infection.