ABSTRACTS

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Matrix metalloproteinase expression in cultured canine trophoblasts

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INTRODUCTION: The factors regulating trophoblast invasion into the canine decidua are not well described. Matrix metalloproteinases play a crucial role in trophoblast implantation and invasion in many species (1). Of these, MMP-2 and -9 are involved in the degradation of the extracellular matrix and cell migration. Trophoblast expression of MMP-2 and -9 has been demonstrated in normal and abnormal human placentas (2). To establish a baseline for future studies investigating placental disorders in dogs, the objective of this research was to determine MMP-2 and -9 expression in cultured canine trophoblasts. We hypothesized that cultured canine trophoblasts would express MMP-2 and -9.

METHODS: Following methods previously described (3), trophoblasts were isolated from three canine placentas using collagenase and trypsin with Percoll density gradient centrifugation. Cells were then cultured in DMEM media (#829415, Gibco-Invitrogen, Carlsbad, CA) at 38ºC with 5% CO2 and grown to 70% confluency on coverslips. Cells were fixed in 70% methanol and expression of MMP-2 (#MS806P0, clone Ab4, Neomarkers, Freemont, CA) and MMP-9 (#RB1539P0, clone Ab9, Neomarkers, Freemont, CA) was confirmed using fluorescent immunohistochemistry (Alexa Flour 488, #A21202, Invitrogen, Carlsbad, CA; Texas Red, #T2767, Invitrogen, Carlsbad, CA). Both MMP antibodies had been used previously for immunohistochemistry in the canine uterus (4). Expression of cytokeratin-7 (#p103620, DAKO, Carpinteria, CA) was to confirm cell type. Hoescht 33342 (#H1399, Invitrogen, Carlsbad, CA) was used to count cells. The average percentage of MMP positive cells for multiple fields was determined for each placenta and reported as the mean±SEM MMP-2 and MMP-9 percent positive. MMP-2 and MMP-9 percent positive cells were compared using a Students t test. The staining intensity and stain localization within MMP positive cells was also noted.

RESULTS: More cultured canine trophoblasts expressed MMP-9 (54.7±3.4%) compared to MMP-2 (40.3±1.8) (p=0.02). However, MMP-2 was more intensely expressed within cells compared to MMP-9 (Figure 1). Although both MMPs were immunolocalized to the cytoplasm, MMP-2 was found in large vesicles, whereas MMP-9 was more diffusely expressed.

DISCUSSION: In trophoblasts from normal human pregnancies, MMP-2 and MMP-9 are expressed at a similar intensity and frequency (75% and 78.5%, respectively) (2). However, it was found that MMP-9 expression was reduced to 15% in trophoblasts from pregnancies complicated with preeclampsia (e.g., those having shallow trophoblast invasion) (2). The canine endotheliochorial placenta is a naturally-occurring shallowly invasive placenta. The lower frequency of MMP positive cells reported in the present study with canine trophoblasts and in the previous study (2) with preeclamptic human trophoblasts could be related to their limited ability to deeply invade the decidua. Activated MMP-2 can activate proMMP-9 (5) but the reverse has not been shown. This may explain why more canine trophoblasts expressed MMP-9 compared to MMP-2. Previous research has demonstrated that the staining pattern in human trophoblasts for MMP-9 is diffusely, whereas the staining pattern for MMP-2 is granular (6). Similar results were found in canine trophoblasts. In addition, we have shown that MMP-2 is more intensely expressed within cultured canine trophoblasts than MMP-9. Future studies using canine placental tissues will investigate the mechanism and significance of this expression, as well as determine if the addition of MMP-2 in culture can induce greater MMP-9 expression.

(2) Sahlfeld LM. Isolation and primary cell culture of canine trophoblasts. Society of Theriogenology Annual Conference. Milwaukee, WI, 2011;358.
Figure 1. MMP-2 (left) and MMP-9 (right) fluorescent immunostaining in cultured canine trophoblasts. Although both MMPs were immunolocalized to the cytoplasm, MMP-2 was found in large vesicles, whereas MMP-9 was more diffusely expressed. MMP-2 was more intensely expressed within each cell compared to MMP-9.