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.\textsuperscript{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.\textsuperscript{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. 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). 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. 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. Although both MMPs were immunolocalized to the cytoplasm, MMP-2 was found in large vesicles, whereas MMP-9 was more diffusely expressed. In trophoblasts from normal human pregnancies, MMP-2 and MMP-9 are expressed at a similar intensity and frequency (75\% and 78.5\%, respectively).\textsuperscript{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).\textsuperscript{2} The canine endetheliocorial 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\textsuperscript{2} with preeclamptic human trophoblasts could be related to their limited ability to deeply invade the decidua. Activated MMP-2 can activate proMMP-9 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 diffuse; whereas the staining pattern for MMP-2 is granular.\textsuperscript{3} Similar results were found in canine trophoblasts. In addition, we have shown that MMP-2 is more intensely expressed within canine cultured 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.