Isolation and primary cell culture of canine trophoblasts
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Introduction
Preeclampsia is a life-threatening condition that affects 5-7% of human pregnancies. Decades of in vitro research in this area have been unsuccessful in learning how to prevent it. In preeclampsia, trophoblasts shallowly invade the endometrial endothelium. This defective trophoblast invasion is detrimental to human pregnancy but represents normal endotheliochorial placentation in dogs. The objective of this research was to establish canine trophoblast cell lines to study in vitro trophoblast invasion and migration as a model for preeclampsia in humans. Cytokeratin-7 is a type II cytokeratin that positively labels human trophoblasts. For this experiment, we hypothesized that cultured canine trophoblasts would also be positive for cytokeratin-7.

Methods
Placentas were removed via hysterotomy from four beagles at 61±1 days from the LH surge (term=65 days). Following methods previously described for isolating human trophoblasts, trophoblasts were isolated using collagenase and trypsin with Percoll density gradient centrifugation. Cells were then cultured in DMEM media (#829415, Gibco-Invitrogen, Carlsbad, CA) at 38°C with 5% CO2 and grown to 70% confluency on coverslips. Cells were fixed in 70% methanol and expression of cytokeratin-7 (#p103620, DAKO, Carpinteria, CA) was confirmed using fluorescent immunohistochemistry (Alexa Flour 488, #A21202, Invitrogen, Carlsbad, CA). Hoescht 33342 (#H1399, Invitrogen, Carlsbad, CA) was used to count cells.

Results
Cellular morphology was consistent with that of trophoblasts; large polygonal cells arranged in a cobblestone configuration. Occasionally, spherical syncytium of cells developed. More than 80% of the cells cultured expressed cytokeratin-7 (Figure).

Conclusion
Using methods for isolating trophoblasts from human placentas, cells isolated from canine placentas had a cellular morphology and immunohistochemistry characteristics consistent with trophoblasts. Future in vitro studies using these cell lines will focus on characterizing canine trophoblast invasion and migration.

Keywords: Canine, cytokeratin-7, immunohistochemistry, preeclampsia, trophoblast

References