ABSTRACTS

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Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon
Determining seeding concentrations for *in vitro* canine cytotrophoblast invasion studies

Gullaba, JM and Kutzler, MA

Department of Animal Sciences, Oregon State University, Corvallis, Oregon, USA, 97331

gullabaj@onid.orst.edu

**INTRODUCTION:** Cytotrophoblasts are specialized epithelial cells of the placenta that proliferate and invade the endometrium at implantation. After formation of a chorioallantois villous labyrinth in early pregnancy, canine cytotrophoblasts continue to advance into the maternal endometrium and remodel the endometrial tissues throughout gestation (1). Little is known about the mechanisms that regulate these processes in dogs, much less the mechanisms that go awry when pathologic cytotrophoblast conditions occur (e.g., subinvolution of placental sites). In humans and rodents, cytotrophoblast invasion has been studied *in vitro* using Matrigel invasion assays (2,3). To yield the desired 50% cell invasion, research in these species has shown that the optimal seeding concentration for Matrigel invasion assays is 250 X 10³ cells/mL (4). The objective of the current study was to determine the optimal seeding concentration of canine cytotrophoblasts for Matrigel invasion assays. Based upon the previously mentioned research in humans, we hypothesized that 250 X 10³ cells/mL would yield the desired 50% cell invasion.

**METHODS:** Canine chorioallantois tissue was collected without the marginal hematoma following elective term C-sections (n=3) and cytotrophoblasts were isolated as described by Sahlfell and coworkers (5). Briefly, cytotrophoblasts were isolated using collagenase and trypsin digestions with Percoll density gradient centrifugation. Four seeding concentrations were compared (0.25, 2.5, 25 and 250 X 10³ cells/mL). Cytotrophoblasts were cultured for 22 hours at 37°C, 5% CO₂ in a protein-free media using the Matrigel invasion assay (8µm pore diameter, polyethylene terephthalate, BD Falcon) using 5% fetal bovine serum as a chemoattractant. Cytotrophoblasts were grown on inserts without Matrigel and treated in the same manner to serve as controls. Cells were stained with Diff Quik® (Harleco, Inc.), and all invading cells were counted at 400X magnification. Percent invasion was determined for each of the conditions by dividing the number of cytotrophoblasts that invaded through the Matrigel by the number of cytotrophoblasts that invade through the control inserts without Matrigel. Results were summarized as mean ± SEM and significance was defined as *p*<0.05.

**RESULTS:** Percent invasion did not differ significantly between the four seeding concentrations (Figure 1). However, the concentration that was closest to the desired 50% cell invasion was 250 X 10³ cells/mL.

**CONCLUSION:** Canine cytotrophoblast invasion is an unexplored field. This study demonstrated that canine cytotrophoblast invasion can be investigated *in vitro* using Matrigel invasion assays. Our results suggest that 250 X 10³ cells/mL is the seeding concentration that would yield approximately 50% cell invasion.

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Figure 1: Influence of seeding concentration on cell invasion. Four cell concentrations (0.25, 2.5, 25 and 250 X 10^3 cells/mL) were seeded onto Matrigel inserts. After a 22-hour incubation, the invading trophoblasts were counted to determine mean±SEM percent invasion.