serum progesterone measurements in less than 30 minutes. This study provides an independent comparison of the POC (Catalyst One, IDEXX) to the current industry standard, CLIA (Immulite-2000, Siemens), used by most veterinary reference laboratories. To assess inter-assay imprecision of POC and agreement of the POC and CLIA results, 100 canine serum samples were analyzed on 3 analyzers (POC-1, POC-2, and CLIA), of which, 74 (POC-1) and 75 (POC-2) results were within POCs' reportable range of 0.2-20 ng/mL and included in the study. To assess intra-assay imprecision, pooled canine serum samples at low (L1), intermediate (L2), and high (L3) progesterone concentrations were analyzed 10 times each on POC-1 and CLIA. Relative to CLIA, POC values had good correlation (POC-1, r = 0.9366; POC-2, r = 0.9438, $p < 0.0001$) and significant positive proportional bias at values > 2 ng/ml. The POC inter-assay coefficients of variation (CVs) were 13.2% (0.2-2.9 ng/ml, 0.6-9.2 nmol/l, L1), 10.0% (3.0-9.9 ng/ml, 9.5-31.5 nmol/l, L2), 7.1% (10.0-20.0 ng/ml, 31.8-63.6 nmol/l, L3), and 11.2% (all samples). The intra-assay CVs for POC (L1, 15.3%; L2, 7.0%; L3, 4.7%) were higher than those for CLIA (L1, 5.89%; L2, 4.89%; L3, 3.44%). The POC had a more rapid increase in serial serum progesterone concentrations in ovulating bitches and had greater imprecision than CLIA. Therefore, caution should be used when interpreting the clinical significance of serum progesterone measurements by the POC as they relate to canine breeding management.

Keywords: Dog, commercial assays, catalyst, accuracy, imprecision

Kisspeptin-10 on in vitro migration of equine chorionic girdle trophoblast cells

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Chorionic girdle (CG) is a specialized component of the equine extraembryonic membranes, composed of rapidly proliferating uninucleated and terminally differentiated binucleated trophoblast cells (uTCs and bTCs, respectively). Gonadotropin-secreting bTCs invade the maternal endometrium to form key structures for pregnancy maintenance known as endometrial cups. Mechanisms that regulate bTC migration and invasion remain elusive. Kisspeptins (Kps), a family of small peptides with 10 (Kp-10) to 54 (Kp-54) amino acids, are highly expressed at the maternal-fetal interface during human and rodent placentation and may inhibit excessive TC invasion. Hence, we aimed to investigate the effect of the equine Kp decapeptide (eKp-10) on CG cell in vitro migration using gap closure and vesicle expansion assays. It was hypothesized that eKp-10 inhibit CG vesicle expansion and the closure of uTC/bTC monolayer gaps. Chorionic girdle was isolated from embryos collected transcervically at 33 - 34 days postovulation (n = 5 mares). Following mechanical dissociation, CG cells were cultured at 37°C in 8% CO2 on serum-supplemented (SM) or serum-free (SFM) medium containing Dulbecco's-modified Eagle's medium. Approximately 500 µm cell-free gaps were formed using silicone inserts (Ibidi®). Once ~ 90% confluency was achieved on both sides of the gap, cells were treated with 0 (control), 1, 10, and 100 µM of eKp-10 and photomicrographs were taken at 0, 6, 12, and 24 hours (n = 8 wells/group) with a Nikon inverted microscope. Concurrently, individualized CG vesicles were transferred from SFM to SM and treated with 0, 0.1, 10, and 100 µM of eKp-10 (n = 20 vesicles/group). Photomicrographs were taken at 0, 12, 24, 36, and 48 hours. Gap widths and vesicle areas were measured by observers blinded to the experimental design using Image J®. Data were assessed via mixed ANOVA and post-hoc Tukey's tests. Significance was set at $p < 0.05$ (JMP Pro 15). Gap closure was slower ($p = 0.04$) in wells treated with 100 µM of eKp-10 compared to control, whereas there was no difference ($p > 0.05$) in closure among control and groups treated with 1 µM or 10 µM of eKp-10). Vesicle expansion occurred in all treatment groups and there was an interaction ($p = 0.0008$) between time and treatment. Within each time point, compared to control, vesicle expansion rate was lower in 0.1 ($p = 0.002$) and 10 µM eKp-10 ($p = 0.03$) at 24 hours, and was also lower ($p = 0.007$) at 36 hours in 0.1 µM and eKp-10. Interestingly, compared to control, expansion rate was higher ($p = 0.04$) in groups treated with 100 µM eKp-10 at 48 hours. Therefore, eKp-10 may affect the migration of subpopulations of CG cells dynamically and in a concentration- and time-dependent manner. Further investigations of Kp expression in the equine maternal-fetal interface and potential role in endometrial cup formation are needed.

Keywords: Mare, endometrial cups, binucleated, trophoblast cells, invasion.