semen was removed after 24 and 48 hours of cooled storage and warmed for 10 minutes at 37°C prior to evaluation of sperm motility parameters using a computer assisted sperm analysis unit (SpermVision®, Minitube of America, Inc., Verona, WI). Data are presented as a mean ± SD. A mixed model was fit to each response variable separately (SAS Institute, Carey, NC). Treatment (40 ml, 20 ml, or 20 ml plus air) was included as a fixed effect. Sample ID was included as a random effect to account for repeat observations on each sample. Tukey adjusted pairwise comparisons were also performed. Data were considered different at $p < 0.05$. Total sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 71.9 ± 14.3, 73.3 ± 13.3 and 76.3 ± 12.5 %, respectively. Total sperm motility values after 48 hours of cooled storage for groups A, B, and C were 65.6 ± 14.1, 65.8 ± 17.3 and 70.9 ± 12.8%, respectively. Progressive sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 66.7 ± 14.9, 67.8 ± 14.4 and 71.7 ± 14.3%, respectively. Finally, progressive sperm motility values for Groups A, B, and C after 48 hours of cooled storage were 60.3 ± 13.6, 60.8 ± 17.8 and 65.7 ± 14.3%, respectively. A difference ($p < 0.05$) in total and progressive motility was detected between Group A and Group C after 24 hours of cooled storage. There were no differences ($p > 0.05$) in total or progressive sperm motility values between aliquots of extended stallion semen in the presence or absence of air after 48 hours of cooled storage. These pilot data suggest that the necessity of removing all air during preparation of a cooled semen dose may not be as absolute as previously considered.

Keywords: Equine, cooled semen, air, sperm, motility

Comparison of nanoparticles and single-layer centrifugation for separation of dead from live stallion sperm

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Artificial insemination with fresh, cooled or frozen semen is commonly used in the equine breeding industry. Poor quality semen with a reduced number of live, motile sperm can lead to lower per cycle pregnancy rates. A study using boar sperm showed improved sperm motility when nanoparticles were used to separate dead from live sperm.[1] The objective was to determine if nanoparticles could separate dead from live stallion sperm. Our hypothesis was that iron-core nanoparticles bind to dead sperm and allow for subsequent separation from live sperm using a magnet. Experiment 1 compared 2 extenders (INRA 96 and TALP-E), 2 incubation temperatures (22 and 37°C) and 6 nanoparticle:sperm ratios (50, 100, 200, 400, 600, and 800 µl of nanoparticle working solution per 100 x 10^6 sperm) using magnetic nanoparticles (ST Genetics, Navasota, TX, US). A research model to mimic a poor-quality ejaculate was made by killing 50% of the sperm by submersion into liquid nitrogen. Experiment 2 compared sperm separation using single-layer centrifugation (SLC) with EquiPure™ (Nidacon International AB, Mölndal, Sweden) versus nanoparticle separation. In both experiments, total and progressive sperm motility, morphology, viability and acrosome status were evaluated. Statistical analysis was performed using one-way ANOVA (data presented as mean ± SD). Values were considered different at $p < 0.05$. Results of Experiment 1: Total and progressive sperm motility were not different between INRA 96 and TALP-E extenders or when incubated at either 22 or 37°C or when using 400 or 600 µl of nanoparticle solution per 100 x 10^9 sperm. Results for Experiment 2: Progressive sperm motility was higher ($p < 0.05$) after SLC (76 ± 9%) than after either nanoparticle treatment (59 ± 12%) or an untreated control (47 ± 5%). In addition, the percentage of viable and acrosome intact sperm was higher after SLC (61 ± 11%) than after nanoparticle treatment (43 ± 3%) or an untreated control (35 ± 3%). There was no statistical difference in sperm morphology among groups. In summary, under the current study conditions based on an induced sperm damage model, single-layer centrifugation performed better than nanoparticles for separating dead from live stallion sperm.

Keywords: Stallion, sperm, nanoparticles, single-layer centrifugation

Reference

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Comparing serum progesterone measurements by a point-of-care analyzer with a chemiluminescent immunoassay in bitch breeding management of the bitch

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Accurate serum progesterone measurements for timing bitches during breeding management is critical for reproductive practice. By monitoring the rise in progesterone during estrus, it is possible to predict the date of ovulation and the peak window of fertility, which is especially important as artificial insemination has become routine to facilitate breeding of animals that are geographically or temporally separated. Although progesterone is a highly conserved molecule across species, laboratory methods for measuring serum progesterone concentrations in the dog vary in accuracy and precision. To measure serum progesterone, chemiluminescent immunoassay (CLIA) has replaced radioimmunoassay as the current standard in the bitch, due to its high correlation and increased practicality. In January 2019, a colorimetric point-of-care (POC) immunoassay was released as an in-clinic diagnostic for quantitative canine

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.