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