Longevity of chilled canine semen with Fresh Express®
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An ideal extender would preserve chilled canine semen quality for up to ten days to account for delays in ovulation in the female, transport time of semen, and availability of the male. Because of reported success of 14-day semen storage using a Tris-egg yolk-glucose extender, we examined longevity provided by Fresh Express® (Zoetis, Florham Park, NJ), a commercially available extender over a period of 14 days by examining motility, viability, and morphology of the spermatozoa and compared the results to those of a second commercially available extender. We hypothesized Fresh Express® would preserve chilled semen resulting in 60% of original motility, 75% of original viability, and negligible changes in morphology at the end of 14 days.

With an estrous bitch present, semen was collected by manual stimulation from five 18± 2 kg, 5- (±2.1) year-old, mixed breed dogs (Day 0). Initial concentration of spermatozoa (cells in 10⁶/ml) and viability were determined using automated fluorescence microscopy (Nucleocounter SP-100; ChemoMetec A/S, Allerød, Denmark). The cells were centrifuged (1400 G, 10 min, 20°C) and Fresh Express® or the second extender added to adjust the concentration to 150 x 10⁶/ml and each sample was split into A and B tubes. Cells were stained with eosin nigrosin and 200 cells counted to assess morphology (detached or dead heads, obvious acrosomal defects, midpiece droplets or defects, kinked, bent or coiled tails). Motility was analyzed by computer-aided sperm analysis (Hamilton Thorne, Beverly, MA) after 20 µl of each sample were diluted to a concentration of 50 x 10⁶/ml. All tubes were refrigerated at 4°C for the remainder of the study. On days 3, 6, 9, 12, and 15, aliquots (A tubes) were removed from each tube, warmed to 20°C, and reassessed as described above. All B tubes were centrifuged (1400 G, 10 min, 4°C), supernatant removed, and fresh extender added before evaluation at the same time points.

Data for motility, viability and morphology were compared using Mann-Whitney statistics. After six days of refrigeration, motility of samples extended in Fresh Express® were 97.4±3.6% of the original value and statistically higher than the motility (64.1±4.6%) using the second extender. The number of viable cells after three days dropped to 91.7±4.7% and 94.7±0.9% of their original values, respectively. Morphological changes using both extenders were minimal and consisted of a less than five percent increase in detached heads and bent tails.

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