Use of a proprietary cell stabilization and preservation solution (HemSol™) to prolong canine semen longevity at room temperature

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Introduction
The industry standard for shipping canine semen is to use an insulated container maintained at 5°C Celsius with semen extended in a commercial milk based semen extender. Effective semen storage at room temperature could decrease the costs of using shipped semen for insemination and increase the convenience in preparing samples. This study examined a proprietary cell stabilization and preservation solution composed of a mixture of cell-impermeable carbohydrates (HemSol™) to prolong room temperature semen longevity.

Hypothesis
Semen extended with HemSol™ at room temperature will have prolonged longevity.

Materials & Methods
Multiple trials were required to determine a potentially optimal admixture for extended canine semen to HemSol™. For each trial, semen was collected via manual stimulation from one dog and was immediately evaluated for concentration, volume, and motility. The initial ejaculate was also evaluated for morphology using an eosin-nigrosin stain. Following evaluation, semen was evenly dispersed into each treatment, and both total and progressive motility were evaluated every 24 hours until total motility was <10%. Any sample that was refrigerated was warmed to room temperature prior to evaluation. All semen used was extended to a concentration of 25 million spermatozoa per milliliter.

Trial 1: Determination of the best commercial semen extender to maintain longevity. Four treatments were evaluated at refrigeration temperature (5°C Celsius) at a 2:1 extender-to-raw semen ratio for:
- ST extender
- LT extender
- Chill 10 extender
- INRA 96 extender

Trial 2: Determination of various admixtures of HemSol™ at room temperature (~22°C Celsius). Semen was admixed with HemSol™ at similar ratios for blood products per manufacturers recommendations:
- 1:1 HemSol™-to-raw semen
- 1:3 HemSol™-to-raw semen
- 1:1 HemSol™-to-extended semen with ST
- 1:3 HemSol™-to-extended semen with ST

Trials 3 and 4: HemSol™ was added in concentrations of 0%, 5%, 10%, 15% and 20% in two separate trials to ascertain the effects of various dilutions of HemSol™ on semen longevity. The 4th trial was done with fresh extender and fresh HemSol™ to confirm the results.

Results
The semen from the dog used was >80% motile and had >80% normal morphology prior to any interventions.

Trial 1: ST semen extender was determined as the best standard semen extender for this dog. It maintained total motility and progressive motility above 10% for eight days with minimal agglutination or morphological defects.

Trial 2: All HemSol™ admixtures decreased progressive motility to below 10% within three days. The control remained well above 50% for both total and progressive motility after three days.

Trial 3 and 4: The motility was best maintained by 5% HemSol™, which remained above 10% total and progressive motility for three days, but was still outperformed by the control which remained above 10% total and progressive motility for five days.

Discussion
This study was performed to see if a proprietary cell stabilization and preservation solution (HemSol™) could potentially be used to make it easier for semen to be shipped at room temperature. This product has been used successfully in storing blood cells and some nucleated cells at room temperature and the company approached LMU about trying this with semen. HemSol™ did preserve sperm plasma membrane integrity when measured by the manufacturer, so further investigation was done to see if it could be used as a semen extender by itself or in conjunction with commercial semen extenders.

- Based on the results of the trials, evidence does not support the use of HemSol™ at room temperature for canine semen storage or shipping since there was a significant reduction in total and progressive semen motility over controls.
- The drastic motility reduction could be caused by incompatibility of sperm cells with a more alkaline HemSol™ pH. Canine semen has a pH from 6.5-7.0, while the pH of HemSol™ is 7.2 +/- 0.2. Osmolarity compatibility also needs examined between HemSol™ and extended canine semen. HemSol™ would not be a good extender at a refrigerated temperature because of the carbohydrate mixture crystalizing.

Conclusion
- The addition of HemSol™, at room temperature, does not enhance room temperature semen longevity.
- Further studies are required to determine if changes in pH, osmolarity, or the carbohydrate composition of HemSol™ will enhance room temperature semen longevity.
- Further studies are required to determine if HemSol™ would be compatible with another dog(s) and/or another species.

References