

SIMPLIFIED HYPO-OSMOTIC TESTING OF CANINE SPERMATOZOA

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Under hypo-osmotic conditions, mammalian spermatozoa undergo a typical reaction caused by the influx and retention of water in spermatozoa with intact membranes. This phenomenon is readily noticed under microscopic examination of spermatozoa displaying curling or coiling of sperm tails, therefore, indicating a biochemically viable sperm membrane. To date, there have been only five publications on the hypo-osmotic testing of canine spermatozoa from three different laboratories; all three laboratories recommend incubating the semen in a hypo-osmotic medium for 30–60 min before conducting semen analyses. The main goal of our study was to compare the “swelling” response of spermatozoa at <1 min with that at 60 min post incubation with the hypo-osmotic medium, and to calculate the correlation between the hypo-osmotic responses with routine semen parameters.

Ejaculates ($n = 15$) were obtained from seven healthy dogs (2–7 years old) of various breeds. Ejaculates were subjected to the following analyses: (1) determination of sperm motility (total and progressive) and concentration using a computer assisted sperm analyzer (CASA; Hamilton-Thorne IVOS); (2) morphology and live:dead ratio using eosin-nigrosin staining; (3) determination of curling/coiling rates of spermatozoa placed into a hypo-osmotic solution (100 mM sucrose solution) at <1 min (HOST < 1 min) and after incubation at 37 °C for 60 min (HOST 60 min). Two hundred spermatozoa were counted for morphology, viability and hypo-osmotic tests.

The degree of association between variables was calculated by the Pearson Product Moment Correlation; responses to HOST < 1min and HOST 60 min were analyzed by ANOVA repeated measures; all analyses were tested with significance level set at $P < 0.50$.

The HOST < 1min and HOST 60 min were positively correlated and tended to increase together with % total motility, % progressively motile sperm, and % live sperm ($P < 0.50$). In our experimental design, we did not detect a significant difference between the responses to the hypo-osmotic test at <1 min and after incubation for 60 min ($P = 0.11$). In the hypo-osmotic testing, we did not detect any spermatozoon with a straight tail (presumably dead) that exhibited any movement (immotile).

The hypo-osmotic testing of canine spermatozoa is a simple, fast and relatively inexpensive test to conduct. Because the response to the hypo-osmotic test was evident as soon as the sperm was added to the hypo-osmotic solution (<1 min), we suggest the hypo-osmotic test may be easily incorporated into a routine canine semen analysis.

Keywords: Canine; Spermatozoa; Hypo-osmotic; Motility; Sucrose