Concentration dependent effect of prostatic fluid on seminal parameters of cooled canine semen

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The aim of this project was to determine the optimal dilution ratio of prostatic fluid (PF) to sperm rich fraction (SRF) for use with cooled, shipped canine semen, by evaluation of in vitro seminal parameters. Our hypothesis was that increasing PF concentration during cooled storage would decrease motility parameters while increasing membrane stability.

The SRF and PF were collected consecutively from four fertile stud dogs (produced a litter in the last six months) on three occasions. The ejaculates were pooled, extended (1:1, v/v) in a tris-based egg yolk extender (EYT, Uppsala Equex I), then divided into four aliquots and centrifuged (700 x g, 8 min). The pellets were re-suspended with EYT modified with 50%, 25%, 10% or 0% PF (Treatment PF50, PF25, PF10 and PF0 = control) and adjusted to a final concentration of 200 x 10^6 cells/mL. Sperm motility (CASA), sperm membrane integrity (HOST) and sperm membrane stability (YO-Pro-1/EthD-1, flow cytometry) were evaluated at 0, 24 and 48 h following storage at 4°C. The data were analyzed using repeated measures ANOVA, mixed linear model (SAS 9.4). Effects of time, treatment and interaction of time x treatment were evaluated with significance level being < 0.05.

A significant effect of time was seen on the variables total motility and progressive motility with no significant differences between treatments. No significant differences of treatment or time on plasma membrane integrity were detected with HOST. There was an effect of treatment on plasma membrane stability: the percentage of cells with a stable plasma membrane (YO-Pro-1 - / EthD-1 -) with PF0 was higher than with PF10 (p < 0.05) or PF25 (p < 0.01) but not PF50 (p = 0.052). Percentage of early unstable cells (YO-Pro-1 + / EthD-1 -) was higher with PF25 than PF0 (p < 0.03) or PF50 (p < 0.01), but not PF10 (p = 0.18). Percentage of late unstable cells (YO-Pro-1 + / EthD-1 +) with PF50 was higher than with PF0 (p < 0.01), PF10 (p < 0.01) or PF25 (p < 0.01). Percentage of necrotic cells (YO-Pro-1 - / EthD-1 +) was higher with PF50 than PF0 (p < 0.03) or PF10 (p < 0.03), but not PF25 (p = 0.51). All other unreported pair-wise comparisons between treatments were not significantly different.

Our data suggest that motility parameters decline over time during storage at 4°C over 48h without significant influence of PF concentration. The presence of a high concentration of PF was shown to have a detrimental effect on plasma membrane stability.

Keywords: Canine semen, prostatic fluid, concentration, cooled storage, seminal parameters