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Canine sperm motility after addition of prostasome-like vesicles at neutral or slightly alkaline pH

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Mammalian seminal plasma contains membranous vesicles (MV) of different origin and composition called prostasome-like vesicles (PV) ejected during ejaculation. In human, they are involved in different functions pH dependent, related to reproduction and fertility. PV have been identified in several animal species where they promote various activities. Their functions in dogs are still controversial and poorly understood. Therefore, the aims of the present study were to evaluate at neutral or slightly alkaline pH the effects of adding purified PV at different concentrations on fresh sperm cells motility characteristics. PV were purified starting from pooled semen as previously described by Zelli et al 2013. The pellet obtained was suspended in buffer with 30 mM Tris-HCl including 130 mM NaCl buffer, adjusted to pH 7.6 with HCL, at a concentration of 800 µg of protein per ml. PV were preserved at -196° C until use. Pooled semen rich fractions from 4 healthy dogs were extended with CaniPro Chill 5 and preserved at room temperature. Sperm cells motility was evaluated using Sperm Vision SAR version 3.5, computer-assisted sperm analysis system (CASA System MOFA Global, Verona, WI). Purified PV were added to pooled semen at time 0 at increasing concentration (1.25, 2.5, 5, 10 and 20 µl) to aliquots of 100 µl of extended semen maintained at room temperature. Control group consisted in 100 µl of extended semen added with 20 µl of buffer. pH and motility characteristics were assessed for each group over time at T0, T 30, T60, T120, T240 and T480 minutes. In general, semen characteristics decreased over time and PV supplementation modified the pattern of some parameters in a concentration-dependent fashion. In particular, a significant decrease in total motility (TM) and progressive motility (PM) was observed in the groups with the highest concentration of PV at 480 minutes. The pH during the experiment showed a decreasing trend in both control and treated groups but without differences (7.33 ± 0.01 at T0 to 7.04 ± 0.01 at T480 in the control sample and 7.3 ± 0.02 at T0 to 7.04 ± 0.01 at T480 in the treated ones). A significantly increased average path velocity (VAP), straight line velocity (VSL) and straightness (STR) was observed at 120 minutes in the treated groups, followed by a significant decrease from 240 to the end of observations. This sustained increase of VAP, VSL and STR could be due to an improved availability of energy substrates originating from the PV. These observations suggest that the addition of PV may affect sperm cells velocity parameters important in the migrations of sperm cells through the female genital tract. In conclusion, the present study demonstrated concentration depend negative effects of PV addition on total and progressive motility of canine semen preserved at neutral and alkaline pH (P <0.05) over time. Furthermore, PV addition, changed the pattern of sperm movements which may represent an additional mechanism involved in sperm cells endometrial attachment and energy preservation before fertilization.
