Removal of seminal plasma by filtration prior to cryopreservation of canine sperm
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Some cryopreservation protocols for dog sperm include removal of seminal plasma by centrifugation. However, centrifugation can potentially induce damage to the sperm of some dogs and requires costly equipment. A sperm filter has been recently developed to remove seminal plasma from stallion semen\(^1\) and its use yielded comparable results to centrifugation. Therefore, our hypothesis was that removal of seminal plasma by filtration would improve canine sperm cryosurvival when compared to centrifugation. Objectives of the study were to determine whether or not removal of seminal plasma by filtration or centrifugation would: 1) yield similar sperm recovery, motility and viability prior to cryopreservation; and 2) affect sperm motility and viability post-thaw. Two ejaculates from each of four dogs were collected, diluted 1:1 (v:v) with freezing extender (CanFreeze - Step 1®; Partnar Animal Health, Port Huron, MI), and divided into two aliquots. Aliquots were then centrifuged (900 x g for 8 min) or filtered through a sperm filter (Botupharma Ltda., Botucatu, Brazil). Sperm were then resuspended in the same extender and frozen according to the manufacturer’s instructions. Sperm concentration, motility and viability were evaluated before and after seminal plasma removal, and after thawing. Total (TM) and progressive (PM) motility were evaluated by Computer-Assisted Sperm Analysis (CASA; Ceros®, Hamilton Thorn Biosciences, Beverly, MA). Sperm concentration and plasma membrane integrity (i.e. viability) were determined using an automated cell counter (NucleoCounter®, ChemoMetec A/S, Denmark) and staining cells with propidium iodide. Data were analyzed by ANOVA for repeated measures and significance was set at P<0.05. Data are expressed as mean percentage ± SEM. There were no differences in sperm recovery (95±4 vs. 91±5), TM (89±2 vs. 89±1) and viability (86±1 vs. 87±1) between centrifugation and filtration methods, respectively. Progressive motility was significantly decreased after removal of seminal plasma by both centrifugation (63±2) and filtration (63±2), compared to pre-processing motility (75±3). Cryopreservation significantly decreased post-thaw TM, PM, and viability, regardless if samples were submitted to centrifugation (48±5, 33±4 and 53±7) or filtration (41±3, 27±2 and 53±6), respectively. Lastly, post-thaw TM and PM were similar between centrifugation (35±9 and 22±7) and filtration (30±6 and 18±5) after incubation at 37 °C for 30 min.. In summary, removal of seminal plasma by filtration was as effective as centrifugation and resulted in similar sperm survival variables post-thaw. Filtration is a potential alternative to centrifugation when equipment is limited. Future studies will focus on the use of filtration for processing semen from dogs whose sperm are more sensitive to cryopreservation.

Reference