Immunohistochemical detection and localization of sperm-associated antigen 6 (SPAG6) in canine spermatozoa

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The highly conserved mammalian homologue, sperm-associated antigen 6 (SPAG6) is correlated with flagellar motility in mice. Mature SPAG6-deficient male mice are infertile and produce sperm with motility and structural defects. Detection of SPAG6 in canine spermatozoa has not been reported. The objective of this preliminary study was to identify SPAG6 on canine spermatozoa and to determine its localization in fresh semen. The rationale for this study is to determine if SPAG6 can be used as a potential fertility biomarker. From four dogs, the sperm-rich second fractions were collected and pooled. SPAG6 was detected with a standardized immunohistochemistry protocol using a primary SPAG6 monoclonal antibody and biotinylated secondary antibody. Detection and localization was determined via an Olympus microscope under light microscopy (200-600X). For each trial, 100-200 sperm cells were evaluated per slide and a minimum of three slides were counted. The study was repeated three times and the results were confirmed by two individuals. In this preliminary study, in fresh canine spermatozoa, SPAG6 was found only in the midpiece section. This result somewhat differs from what is found in the mouse: localization on SPAG6 at the principal piece, midpiece and head sections. Since it is known that SPAG6-deficient mice have motility and structural defects, using SPAG6 as a fertility biomarker in the canine may prove to be a useful tool for the assessment of canine fertility. Further studies are forthcoming to compare detection and localization of SPAG6 in fresh, chilled, and frozen semen.

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