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JOURNAL ARTICLE

Localization of clusterin on freeze-preserved bull spermatozoa before and after glass wool-sephadex filtration

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Clusterin is a major protein in bull reproductive tract secretions and sperm membrane extract. A polyclonal antibody was produced against clusterin from bull cauda epididymal fluid (CEF) and used for the localization of the protein on bull spermatozoa. Immunoblotting of unreduced bovine samples showed that the anticlusterin antibody reacted with a protein of approximately 94- to 100-kd in rete testis fluid (RTF), a approximately 57- to 76-kd protein in CEF, and with a approximately 57- to 60-kd protein from cauda epididymal sperm membrane extract. The antibody also reacted with stallion RTF and both ram CEF and RTF at relative molecular weights (Mr) that were consistent with the anticipated size of clusterin in these species. Less intense immunostaining was observed for a protein of about 2 times the predicted size of clusterin in unreduced ovine RTF, suggesting the presence of multimers of clusterin in ovine RTF. Also, a dimeric clusterin-sized protein was detected in reduced bovine CEF, suggesting the presence of unprocessed clusterin in bovine CEF. By immunofluorescence, clusterin was detected on only a small fraction of bull spermatozoa, which were morphologically abnormal. Neither permeabilization nor the method of dilution affected the reactivity of the antibody with spermatozoa ($P > .05$). Average clusterin-positive spermatozoa (CPS) in unpermeabilized, permeabilized, abruptly diluted, and gradually diluted semen were 10.1%, 11.3%, 15.0%, and 14.4%, respectively. CPS were eliminated from semen after filtration through glass wool-Sephadex (GWS) columns. Average CPS in unfiltered and filtered semen were 14.3% and 1.1%, respectively. We conclude that sperm clusterin in bull semen is associated with morphologically abnormal spermatozoa and that clusterin is implicated in the process of abnormal spermatozoa trapping in GWS columns. We suggest that the fraction of CPS in bull semen is a potential marker for poor semen quality.