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Reproductive tract secretions and bull spermatozoa contain different clusterin isoforms that cluster cells and inhibit complement-induced cytolysis

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Clusterin from bull rete testis fluid (RTF), cauda epididymal fluid (CEF), and octyl-beta-D-glucopyranoside extract of cauda epididymal sperm (CES) was identified and characterized using monoclonal and polyclonal antibodies (Abs) developed against ram clusterin and a beta-subunit-specific oligopeptide of porcine clusterin. One-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blotting showed that bovine RTF clusterin had dimeric and monomeric molecular weights (M(r)s) of approximately 94 kDa and of 42 and 43 kDa, respectively. Clusterin in CEF and CES had similar dimeric M(r)s (74 kDa). Reduced CEF clusterin appeared as three monomers (M(r)=40, 39, and 38 kDa), whereas reduced CES clusterin appeared only at M(r)40 kDa. Enzymatic deglycosylation resulted in similar M(r)s of clusterin from RTF, CEF, and CES. The M(r) of RTF clusterin decreased from 94 kDa to 51 kDa, indicating a carbohydrate content of 45%. After deglycosylation, the M(r) of the CEF clusterin decreased from 74 kDa to two distinct bands at 51 and 50 kDa (with carbohydrate contents of 31 and 32%, respectively), suggesting that two isoforms of the heterodimeric protein are present because of the two isoforms of the alpha-subunit. Under nonreduced conditions, a beta-subunit-specific Ab reacted with M(r) of 36-38 kDa, indicating the existence of free clusterin beta-subunits in CES. RTF, CEF, and CES extracts all caused mouse fibroblastic L-cell aggregation. CEF cell aggregation was inhibited by Hyb-17 Ab but not by other Abs. Both RTF and CEF caused a dose-dependent inhibition of complement-induced cytolysis, although RTF clusterin was more potent than CEF clusterin. We conclude that several isoforms of clusterin occur in the bull reproductive tract and that the variation in carbohydrate content among these isoforms may affect the biological or functional activity of the protein.

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