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JOURNAL ARTICLE

Anti-bull sperm monoclonal antibodies: II. Binding changes during capacitation and influence on sperm-zona interactions in vitro

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Anti-bull sperm monoclonal antibodies (mAbs), generated against intra-acrosomal and surface antigens, were evaluated for their functional significance. In experiment I, the influence of mAbs on the bovine sperm-oocyte interaction in vitro was tested on a total of 493 oocytes in either three or four replicate trials. Although the number of sperm bound per zona increased significantly over untreated control samples (23.6 +/- 5.6 vs. 10.0 +/- 2.4, mean +/- standard error [SE]; $P < 0.001$) in the presence of one surface-reacting mAb, other mAbs had no effect. Experiment II was designed to determine if the mAbs would detect capacitation-related changes of bull sperm in vitro. Bull sperm were incubated in capacitation medium (supplemented Tyrode's medium [TALP] plus 10 micrograms/ml heparin) for up to 4.5 hours. At 0 and at 4 hours, mAbs in hybridoma culture supernatant were incubated for 30 minutes with sperm, labeled with fluorescein isothiocyanate (FITC)-conjugated secondary antibody, and processed for indirect immunofluorescence assay. Four mAbs specific to intra-acrosomal antigens exhibited a time-dependent increase ($P < 0.05$) in binding to bull sperm incubated under capacitation conditions. In contrast, the binding of the mAbs specific to surface antigens significantly decreased ($P < 0.05$) after 4 hours in the presence of heparin. Sperm viability did not change during the 4-hour period. In experiment III, mAbs specific to intra-acrosomal antigens were evaluated to assess bull sperm acrosomal status following lysophosphatidylcholine-induced acrosome reaction. A significant decrease ($P < 0.01$) in mAb binding following the induced acrosome reaction was observed with all the mAbs; it was highly correlated ($r > \text{or} = 0.85$; $P < 0.01$) with *Pisum sativum* agglutinin binding and Giemsa staining. The results suggest that some of the mAbs are potential biological markers for bull sperm surface changes associated with capacitation and the acrosome reaction in vitro.

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Vitro Fertilization

Biol Reprod, June 1, 1999; 60(6): 1285 - 1291.

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