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JOURNAL ARTICLE

Nanovid microscopy for assessing sperm membrane changes induced by in vitro capacitating and acrosomal reacting procedures

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This study was to verify the usefulness of Nanovid microscopy techniques for evaluating induced modifications in bovine spermatozoal membranes. Frozen thawed bovine sperm were labeled with 20-nm colloidal gold particles bound to concanavalin A (ConA) or heparin ligands. Sperm membrane changes were induced in vitro by capacitating and acrosome-reacting procedures. Capacitation was induced by incubation with 10 micrograms/ml of heparin for 4 hours at 37 degrees C, 5% CO2, and high humidity. Membrane changes associated with the acrosome reaction were induced by addition of lysophosphatidylcholine (100 micrograms/ml) and incubation for 15 minutes at 37 degrees C, 5% CO2, and high humidity. Gray intensity (black = 0; white = 255) of sperm (ONCELL) and background (OFFCELL) were evaluated with computer-enhanced videomicroscopy with either differential interference contrast (DIC) or Nanovid optics. A high gold concentration on a membrane region produced blacker video pictures with Nanovid microscopy. Gray intensity of video pictures of a region with little or no gold would have a gray intensity equal to or greater than that of the background, that is, toward white. Weighted least squares methods were used to analyze ONCELL data using OFFCELL as a covariate. In experiment 1, ONCELL intensities of cells labeled with ConA-gold complex were lower than those labeled with heparin-gold at the same treatment level. In experiment 2, ONCELL intensity decreased as the concentration of heparin-gold increased from 0 to 0.041 microgram/microliter heparin. ONCELL intensity significantly decreased after sperm were treated with the highest heparin-gold level and acrosome reacted.(ABSTRACT TRUNCATED AT 250 WORDS)