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Assessment of the acrosomal status of ram spermatozoa by RCA lectin-binding and partition in an aqueous two-phase system

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The acrosome reaction is an important marker for sperm function. Because different laboratory techniques may be used to detect this exocytotic process, the objective of this study was to investigate the use of fluoresceinated lectins to assess the acrosomal status of nonpermeabilized ram spermatozoa. In addition, we used centrifugal countercurrent distribution (CCCD) in an aqueous 2-phase system to assess the sperm surface modifications associated with the acrosome reaction by observing changes in their partition behavior. We analyzed the binding of 5-fluorescein isothiocyanate (FITC)-conjugated lectins to ram sperm to select a lectin that bound preferentially to the acrosomal region, which would allow differentiation of acrosome-intact from acrosome-damaged ram spermatozoa. Ricinus communis agglutinin (RCA) bound intensely to the anterior and weakly to the equatorial acrosomal regions. Acrosomal labeling changed when spermatozoa were induced to acrosome-react with calcium ionophore A23187. RCA acrosomal labeling significantly increased ($P < .0001$) after incubation (84% versus 28% in control samples). To determine if RCA lectin labeling could be used to assess the acrosomal status of fresh ram spermatozoa in suspension, we compared the percentage of acrosome-reacted sperm detected by the carboxyfluorescein diacetate/propidium iodide (CFDA/PI) double-fluorescent staining with the percentage detected by FITC-RCA labeling. The incidence of acrosome-reacted spermatozoa detected by CFDA/PI was not significantly different ($P = .704$; 13 comparisons in 6 different experiments) from the incidence of spermatozoa detected by FITC-RCA staining. The evaluation of the spontaneous acrosome reaction by RCA labeling (5.83%) was not significantly different ($P = .644$) from that assessed by CFDA/PI (6.88%). The percentage of induced acrosome reactions detected by CFDA/PI staining (56%) significantly correlated ($P < .0001$; $r = 0.876$) with that detected by RCA labeling (56.67%). We simultaneously carried out a comparative CCCD in an aqueous 2-phase system to analyze sperm surface changes associated with the acrosome reaction. Results revealed that sperm surface hydrophobicity decreased in samples that had been incubated with ionophore compared with the untreated-control samples. Likewise, RCA binding after CCCD showed that all acrosome-reacted cells were stained, whereas only 42% of cells were lectin-labeled in the untreated semen sample. This change in lectin reactivity of acrosome-reacted spermatozoa signals the presence of some deep membrane or intracellular residues that would affect partitioning. Therefore, the FITC-RCA-labeling procedure can be used to accurately assess the acrosomal status of ram

spermatozoa in suspension.

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