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Annexin V Binding and Merocyanine Staining Fail to Detect Human Sperm Capacitation

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The signaling pathways that characterize the process of capacitation of human spermatozoa are still largely unknown. Modifications in the lipid architecture of the sperm plasma membrane have been described in spermatozoa from different species, including translocation of phosphatidylserine (PS) from the inner to the outer leaflet and increased phospholipid disorder in the membrane. In human spermatozoa, however, results of PS exposure are controversial. In the present study, we used flow cytometry to investigate both membrane PS exposure by Annexin V (Ann V) binding and lipid disorder by merocyanine 540 (M540) staining, in swimup–selected live spermatozoa after incubation in conditions leading to capacitation. Our results indicate that neither probe is able to detect capacitation-related membrane modifications. Investigation of the nature of PS exposure and M540-positive live cells was then carried out. We found that M540 stains elements devoid of nuclei are present in seminal plasma. Live PS-exposing cells were mainly represented by damaged spermatozoa as revealed by the occurrence of a negative correlation between PS exposure and normal morphology and motility in unselected samples. The same cells were also positive for M540. These results demonstrate that Ann V and M540 binding in human sperm samples mainly detects cells with early membrane degeneration as well as dead cells, which is in agreement with findings obtained for somatic cells in which the two probes recognize cells with a damaged membrane due to the apoptotic process.

Key words: FACScan, membrane architecture, phosphatidylserine translocation

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