Journal of Andrology, Vol 21, Issue 2 238-249, Copyright © 2000 by The American Society of Andrology

JOURNAL ARTICLE

Measurement of intracellular calcium concentration and plasma membrane potential in human spermatozoa using flow cytometry

I. A. Brewis, I. E. Morton, S. N. Mohammad, C. E. Browes and H. D. Moore

Department of Molecular Biology and Biotechnology, University of Sheffield, United Kingdom. i.brewis@sheffield.ac.uk

We report 2 novel approaches using flow cytometry to measure intracellular calcium concentration and plasma membrane potential in human spermatozoa. Both approaches have the potential to measure different responses in subpopulations of cells, which is particularly useful when studying heterogeneous populations such as human spermatozoa. Intracellular calcium concentration ([Ca2+]i) was measured using the probe indo-1/AM. This allowed measurements to be made that were independent of variation in cell size and dye loading. It also enabled dead cells to be directly identified and excluded from the analyses without the need for counterstaining. Mean basal [Ca2+]i was determined as 50 nM (25-75 nM range) and, in response to the agonist progesterone (20 microM), this increased transiently to 195 nM (125-285 nM range) before declining to approximately half the maximal level within 2 minutes (values in parentheses correspond to the range of values typically found within a sperm population from 1 sample). These results are comparable with previously published data on whole sperm populations. Sperm membrane potential (VM) was assayed using the probe DiOC6(3). In carefully controlled experiments, a marked depolarization of the plasma membrane potential of capacitated spermatozoa was observed in response to progesterone (20 microM). Following in vitro capacitation, the sperm plasma membrane potential became hyperpolarized compared with the noncapacitated state. Therefore, this technique may be used to assay for sperm capacitation in vitro.

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