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JOURNAL ARTICLE

Analysis of the responses of human spermatozoa to A23187 employing a novel technique for assessing the acrosome reaction

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Protocols for the use of A23187 in assessing the ability of human spermatozoa to acrosome-react and exhibit sperm-oocyte fusion have been developed and the results compared in two independent cohorts of infertile patients. Both bioassays were found to depend upon such factors as the dose and formulation of A23187, the duration of exposure, and the amount and type of protein used to supplement the medium. An optimal protocol for the hamster oocyte penetration test comprised a 3-hour exposure to 1.25-2.5 μM ionophore and gave penetration rates of 93.2 \pm 3.2% (11.26 \pm 1.27 sperm/egg) for a group of 33 fertile donors compared with 63.0 \pm 5.4% (4.73 \pm 0.81 sperm/egg) for a cohort of 56 patients consulting for infertility ($P < 0.001$). Higher doses (5.0-10.0 μM) of A23187 caused an inhibition of sperm-oocyte fusion in association with a loss of motility, although the integrity of sperm plasma membrane did not appear to be compromised and high rates (approximately 80%) of acrosome reaction were observed. A protocol for assessing the ability of viable human spermatozoa to acrosome-react in response to A23187 was developed, employing a fluorescein-conjugated lectin in concert with the hypoosmotic swelling test, which gave values of 20.1 \pm 2.6% and 13.6 \pm 1.6% for groups of fertile donors ($n = 29$) and infertile patients ($n = 32$) respectively ($P < 0.05$). Although only acrosome-reacted spermatozoa were capable of fusing with zona-free hamster oocytes, there was no significant correlation between the proportion of acrosome-reacted cells and the levels of sperm-oocyte fusion observed in two independent groups of patients, indicating that these bioassays are measuring different aspects of human sperm function. These results have implications for the way in which the responses of human spermatozoa to ionophore treatment are quantified and interpreted.

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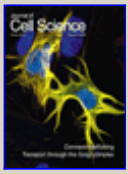
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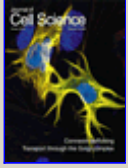
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