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JOURNAL ARTICLE

Human sperm capacitation induced by biological fluids and progesterone, but not by NADH or NADPH, is associated with the production of superoxide anion

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Recent evidence indicated that human sperm capacitation is associated with an increased production of superoxide anion (O2.-). To further study the role and importance of O2.- in capacitation, we investigated whether the O2.- generation is a general feature of capacitating spermatozoa, irrespective of the inducer used, and is correlated with capacitation levels and increased tyrosine phosphorylation of two sperm proteins (p105/p81). We also studied the time courses of O2.- production and action. Percoll-washed human spermatozoa were incubated in Ham's F-10 medium, supplemented or not supplemented with various capacitation inducers and in the presence or absence of superoxide dismutase (SOD). Sperm capacitation was measured by induction of the acrosome reaction with lysophosphatidylcholine, O2.- production was measured by chemiluminescence, and tyrosine phosphorylation was measured by immunodetection after electrophoresis and western blotting of sperm proteins. Progesterone and ultrafiltrates of human fetal cord serum, follicular fluid, and seminal plasma individually promoted sperm generation of O2.-, tyrosine phosphorylation of p105/p81, and capacitation. Fetal cord serum ultrafiltrate triggered a fivefold higher O2.- production than the other inducers (1,700 +/- 300 and 300 to 400 mV/10s/8 x 10(6) cells, respectively), a phenomenon possibly associated with the higher potency of this fluid to promote sperm hyperactivation. The production of O2.- by spermatozoa was rapid and transient. SOD prevented sperm capacitation triggered by the inducers mentioned above, but only when SOD was added at the beginning of incubation, and not after 30 minutes, indicating that the O2.- initiates a chain of early events leading to sperm capacitation. NADH and NADPH (5 mM) triggered sperm capacitation and phosphorylation of p105/p81, but these processes were not prevented by SOD or catalase, nor were they associated with an increased O2.- production. Therefore, these cofactors appeared to act by mechanisms different from those used by the other inducers studied. The sperm enzyme responsible for the O2.- generation may be very different from the NADPH oxidase of neutrophils.

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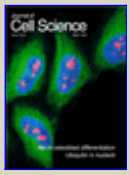

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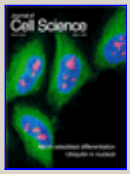

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