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Viability and Protein Phosphorylation Patterns of Boar Spermatozoa Agglutinated by Treatment With a Cell-Permeable Cyclic Adenosine 3',5'-Monophosphate Analog

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Boar spermatozoa become agglutinated with one another at the head when their intracellular cyclic adenosine 3',5'-monophosphate (cAMP)-signaling cascades are activated in the head. The aim of the present study is to examine viability and protein phosphorylation patterns of cAMP-dependently agglutinated boar spermatozoa. Ejaculated spermatozoa were washed and then incubated in a modified Krebs-Ringer HEPES medium containing polyvinyl alcohol (mKRH-PVA) plus 0.1 mM Sp-5,6-dichloro-1-ß-D-ribofuranosyl-benzimidazole-3',5'-monophosphorothioate (cBiMPS, a cell-permeable cAMP analog) at 38.5°C up to 180 minutes. Aliquots of the sperm suspensions were recovered after various incubation periods and then used to examine the state of agglutination, the viability by SYBR14-PI staining and motility assay, and the state of protein phosphorylation by Western blotting and indirect immunofluorescence. In the control samples incubated without cBiMPS for 180 minutes, less than 30% of the total spermatozoa were agglutinated with one another at the heads, and more than 70% of the agglutinated spermatozoa were propidium iodide (PI)-positive (dead). However, the incubation with cBiMPS rapidly increased the percentages of head-to-head agglutinated spermatozoa to approximately 60% within 30 minutes, but did not significantly change them thereafter. In the samples incubated with cBiMPS for 180 minutes, moreover, the percentages of PI-positive cells of the agglutinated spermatozoa (approximately 30%) were significantly lower than those obtained in the control samples (more than 70%). This result was supported by the observation that the percentages of motile cells of the agglutinated spermatozoa were much higher in the samples incubated with cBiMPS for 180 minutes than in the control samples incubated without cBiMPS. As revealed by Western blotting and indirect immunofluorescence, cBiMPS-induced serine/threonine phosphorylation of the proteins (eg, >220 kd, 220 kd, 180 kd, 84 kd, and 54 kd) appeared mainly in the connecting and principal pieces of both agglutinated and free spermatozoa within 30 minutes, and additional phosphorylation occurred in the middle piece later than 30 minutes. Moreover, tyrosine phosphorylation of the proteins (eg, >220 kd, 190 kd, 93 kd, 59 kd, 54 kd, and 32 kd) was induced intensely in the connecting and principal pieces and moderately in the middle piece of almost one half of the agglutinated spermatozoa after incubation with cBiMPS for more than 30 minutes, but rarely in those of the free spermatozoa. These findings are consistent with the following suggestions: activation of the cAMP-signaling cascades leads to rapid (within 30 minutes) head-to-head

agglutination in live spermatozoa; rapid (within 30 minutes) protein serine/threonine phosphorylation in the connecting and principal pieces of both cAMP-dependently agglutinated and free spermatozoa and subsequent (later than 30 minutes) phosphorylation in the middle piece of them; and slow (later than 30 minutes) protein tyrosine phosphorylation in the connecting, middle, and principal pieces of the cAMP-dependently agglutinated spermatozoa. Based on these suggestions, we conclude that many of cAMP-dependently agglutinated spermatozoa are live cells in which cAMP-signaling cascades leading to protein serine/threonine and tyrosine phosphorylation are activated in the whole flagellum.

Key words: Serine/threonine phosphorylation, tyrosine phosphorylation, hyperactivation