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# A Cyclic Adenosine 3',5'-Monophosphate Stimulates Phospholipase CY1-Calcium Signaling via the Activation of Tyrosine Kinase in Boar Spermatozoa

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The aim of this study was to reveal a downstream part of the intracellular signaling that is mediated by cyclic adenosine monophosphate (cAMP)-dependent tyrosine kinases, including spleen tyrosine (Y) kinase (SYK), in boar spermatozoa. Ejaculated spermatozoa were incubated with cBiMPS (a cell-permeable cAMP analog; 0.1 mM) at 38.5°C for 180 minutes and then used for

Western blot and indirect immunofluorescence. Incubation of spermatozoa with cBiMPS induced tyrosine phosphorylation at the linker region of SYK (which was essential to binding to phospholipase C [PLC] $\gamma$ 1) in the connecting and principal pieces, but the tyrosine phosphorylation was abolished by the addition of H-89 (a protein kinase A [PKA] inhibitor; 0.01-0.1 mM). Moreover, the cAMP-dependent tyrosine phosphorylation was also induced at the key regulatory residue of PLC $\gamma$ 1 in the same segments of spermatozoa, but it was inhibited by the addition of herbimycin A (a tyrosine kinase inhibitor; 5  $\mu$ M). These results suggest that the sperm cAMP-dependent tyrosine kinases, including SYK, are linked to the activation of PLC $\gamma$ 1. Indirect immunofluorescence clearly detected both inositol 1,4,5-trisphosphate (IP<sub>3</sub>) receptor and calreticulin in the connecting piece, indicating the presence of internal calcium store. Cell imaging with fluo-3/AM (a cell-permeable Ca<sup>2+</sup> indicator) showed that incubation of spermatozoa with cBiMPS increased intracellular free calcium in the middle piece, but that it was reduced by the addition of U-73122 (a PLC inhibitor; 0.02 mM). Based on our findings, we conclude that the connecting piece of boar spermatozoa possesses the PLC $\gamma$ 1-IP<sub>3</sub> receptor-calcium signaling that is triggered by cAMP and mediated by PKA and herbimycin A-sensitive tyrosine kinases, including SYK.

Key words: Sperm, cAMP, SYK, PLC, calcium store

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