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Involvement of Cytoplasmic Free Calcium in Boar Sperm: Head-to-Head Agglutination Induced by a Cell-Permeable Cyclic Adenosine Monophosphate Analog

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When boar spermatozoa are incubated in a medium designed for in vitro fertilization, many of them become agglutinated at the acrosomes. We previously reported that bicarbonate and cyclic adenosine 3',5'-monophosphate (cAMP) promote agglutination. The aim of the present study is to examine the role of cytoplasmic free Ca^{2+} in boar sperm agglutination induced by a cell-permeable cAMP analogue. Spermatozoa were collected from five mature boars, washed, and resuspended in a modified Krebs-Ringer-Hepes solution lacking calcium chloride. The sperm suspensions were incubated in a water bath (38.5°C) for 60 minutes and were then used to determine the percentages of head-to-head agglutinated spermatozoa. Percentages of head-to-head agglutinated spermatozoa in the samples rose significantly after incubation, from 28% to 61%-62%, after adding to the medium a cell-permeable, phosphodiesterase-resistant cAMP analogue (cBiMPS, 10 µM) or an adenylyl cyclase stimulator (sodium bicarbonate, 5 mM) plus a cell-permeable phosphodiesterase inhibitor (IBMX, 25 µM). However, the promoting effects of these reagents were blocked when spermatozoa were pretreated with a cell-permeable Ca^{2+} chelator (BAPTA-AM, 25 µM), whereas the same pretreatment with a cell-impermeable Ca^{2+} chelator (BAPTA, 25 µM) had almost no influence on sperm agglutination. Adding thapsigargin, a potential Ca^{2+}-ATPase inhibitor, to the medium raised the percentages of agglutinated spermatozoa in a concentration-dependent manner for concentrations up to 4 µM. When 4 µM thapsigargin and 10 µM cBiMPS were examined for their effects on free Ca^{2+} levels in sperm heads by using a cell-permeable Ca^{2+} indicator (fluo-3/AM), the samples incubated with both or either of these reagents contained many head-to-head agglutinated cells that exhibited intense fluorescence in the heads. In control samples incubated without these reagents by contrast, most spermatozoa were free (unagglutinated) cells and characterized by almost no or only slight fluorescence in the heads. Moreover, morphological observation of Giemsa-stained preparations revealed that most agglutinated spermatozoa possessed darkly stained acrosomes, which distinguished them from acrosomereacted spermatozoa. This indicated that the sperm agglutination was not a result of the acrosome reaction. Furthermore, with indirect immunofluorescence of Ca^{2+}-ATPases, the mouse monoclonal antibody to this enzyme demonstrated high affinity to the acrosomes of permeabilized spermatozoa. Based on these results, we conclude that cytoplasmic free Ca^{2+} is

involved in sperm head-to-head agglutination induced by a cAMP analogue.

Key words: Capacitation, BAPTA, thapsigargin, fluo-3, Ca^{2+}-ATPase

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