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## JOURNAL ARTICLE

# Calpain and calpastatin are located between the plasma membrane and outer acrosomal membrane of cynomolgus macaque spermatozoa

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Mammalian sperm must undergo an acrosome reaction prior to penetration of the zona pellucida and subsequent fusion with an oocyte. Sperm gain the capability to acrosome react after a period of capacitation, which primarily involves biochemical changes in the sperm membranes. The morphological events of the acrosome reaction have been well-documented, but the underlying cellular mechanisms that regulate capacitation and the acrosome reaction remain unclear. Antibodies to the 2 ubiquitous calpains, mu and m, as well as the small subunit, which associates with both calpains, were localized at the ultrastructural level to the region between the plasma membrane and the outer acrosomal membrane of cynomolgus macaque sperm. After the acrosome reaction, all of the anti-calpain antibodies labeled the acrosomal shroud, suggesting that calpains are located throughout the cytoplasmic area between the 2 outer sperm membranes. Calpastatin is an endogenous modulator of calpain activity and is also localized within the same cytoplasmic region as calpains. The antibodies used for ultrastructural localization were also used to probe Western blots of sperm extracts. Antibodies to either the mu- or m-calpain recognized an 80-kd protein, which is similar to the molecular weights of other ubiquitous calpains described. The small subunit (30 kd) was also recognized with a specific monoclonal antibody. An antibody to calpastatin recognized a major band at 78 kd and a lighter band at 45 kd, while the antibody to the testis-specific isoform of calpastatin (TCAST) recognized a 110-kd protein. We hypothesize that this cysteine protease system may be functional in cynomolgus macaque sperm during capacitation, the acrosome reaction, or both.

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