

ABSTRACT

The Rab-2A protein belongs to the Rab family of monomeric G-proteins involved in the regulation of intracellular vesicular transport. Although several studies describing the role of Rab-2A protein in mammalian sperm have been published, the exact localization and function of this protein in male gametes have not been fully understood yet. In the diploma thesis, we were the first to describe the presence of Rab-2A in human sperm using 5C5, produced by the Laboratory of Reproductive Biology. Using the 5C5 antibody, we were able to identify several Rab-2A isoforms with molecular weights of 26, 24, 22, and 18 kDa in the human sperm lysate, confirmed by mass spectrometry analysis. Using the indirect immunofluorescence method, we localized Rab-2A in the acrosome area of both ejaculated and capacitated sperm. We noticed that Rab-2A leaves the sperm during the acrosomal exocytosis. We also found a reduced Rab-2A protein level in sperm of patients with various pathologies of ejaculate. Based on studies describing the role of Rab-2A in acrosome biogenesis and the presence of Rab-2A in bovine sperm perinuclear theca, we tried to detect Rab-2A in this protein layer in human sperm. However, the chosen method is apparently not applicable to human sperm and isolation has failed. Additionally, we demonstrated the presence of Rab-2A in secretory vesicles derived from human seminal plasma, where we detected only 24 and 22 kDa isoforms. Although we were not successful in confirmation of the presence of Rab-2A on the surface of human sperm, we found binding activity to zona pellucida glycoproteins in several sperm proteins, including low-molecular-mass proteins (18 and 30 kDa), that could correspond to Rab-2A isoforms recognized by the 5C5 in human sperm extracts.