Characterization of Na^+/H^+ Exchanger (NHE11) by Immunofluorescence and High-Resolution Microscopy in Porcine Sperm

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Na^+/H^+ exchangers (NHEs) encode a family of 13 proteins and are vital pH regulators in numerous cells types. Specifically, sperm function is particularly dependent on pH variations, and consequently, NHEs have been suggested to play significant roles in regulating the intracellular pH of these cells throughout fecundation process. In this context, the presence of the NHE11 exchanger has been studied in human and rat sperm, but there is no information about the presence and location of this exchanger in boar sperm. Therefore, the objective of this study was to characterize the immunolocalization of the NHE11 exchanger in boar sperm under different physiological conditions using fluorescence and high-resolution microscopy. The sperm-rich fraction was obtained from tested fertile Piétran boars (n=8). The sperm were capacitated by swim-up and the induction of the acrosome reaction *in vitro* was performed using the calcium ionophore A23187. In each sperm physiological condition, the NHE11 exchanger was characterized qualitatively by immunofluorescence using confocal microscopy and quantitatively by immunodetection using colloidal gold particle through the Field Emission Scanning Electronic Microscope (FESEM). Furthermore, in the fluorescence study, the integrity of the acrosome was evaluated by co-labeling using the lectin *Pisum sativum agglutinin*. As a result, a total of five fluorescence patterns were identified in the sperm head based on the location of the NHE11 exchanger: Pattern 1, periacrosomal region and acrosome region; Pattern 2, periacrosomal region with equatorial band; Pattern 3, equatorial region; Pattern 4, faint labelling; and Pattern 5, without labeling. Specifically, Pattern 1 was mostly significant in uncapacitated and capacitated sperm. While, the rest of the patterns were preferentially visualized in those sperm that had completed the acrosome reaction. In parallel, the data obtained by FESEM confirmed the presence and localization of the exchanger NHE11 in the different physiological conditions of porcine sperm. These data show for the first time the presence and localization of the exchanger NHE11 in porcine sperm. Overall, the detection of this exchanger could indicate a relevant role in the regulation of the capacitation process, as well as during the acrosome reaction or sperm/egg fusion events.

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