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Modulation of voltage-gated sodium channels induces capacitation in bull spermatozoa through phosphorylation of tyrosine containing proteins

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ABSTRACT

In our previous study, we have reported the molecular presence of Na_v 1.8 in bull spermatozoa and its potential involvement in regulation of sperm functions. With the selective blocking of Nav 1.8 using A-803467, alterations in sperm functions were observed, therefore, we envisaged of investigating the involvement of Na_v in regulating sperm function and the mechanism(s) involved in it using veratridine, a selective opener of Na_v channels. Forty ejaculates were collected from four Hariana bulls and semen samples were pooled in view of the non-significant variations between the different ejaculates. Treatment of sperm cells with veratridine (6, 8, and 10µM) resulted in concentration- and time- dependent increase in forward progressive sperm motility and it persisted up to 6h. However, hyperactive motility was induced by veratridine at higher concentrations (8 and 10 µM) and after 2h of incubation, which was confirmed by subjective assessment followed by chlortetracycline staining showing the increased B-pattern spermatozoa, and thereby suggesting the involvement of Na_v in regulation of capacitation in spermatozoa. To substantiate the functional study observations especially veratridine-induced capacitation, immunoblotting and indirect immune fluorescence assays were performed for detection of the tyrosine-phosphorylated proteins. The immune blot study revealed the presence of five tyrosine phosphorylated proteins, namely- p17, p30, p54, p90 and p100. The p17 protein showed the highest band intensity compared to other protein bands indicating its potential involvement in the process of capacitation. Immunolocalization study revealed positive immunoreactivity for tyrosine phosphorylated proteins in the middle piece, post acrosomal region (high fluorescence) and tail of the spermatozoa (low fluorescence). From the results of present study, it is evident that activation of Na_V by veratridine, especially at higher concentrations, induced capacitation which is evidently mediated through phosphorylation of the tyrosine containing proteins localized in the post acrosomal regions, middle piece and tail of the spermatozoa. However, further studies will help in unraveling the involvement of Na_v and other ion channels regulating different physiological functions of sperm.

Key Words: Na_v 1.8; A-803467; bull; capacitation; spermatozoa; immunolocalization