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Molecular characterization of voltage-gated potassium channel (Kv) and its importance in functional dynamics in bull spermatozoa

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ABSTRACT

Present study was undertaken to characterize the voltage gated potassium channel (K_v 1.1) in bull spermatozoa using sixty four ejaculates collected from four Hariana bulls. Functional characterization was undertaken using a selective blocker of Kv channel, 4-Aminopyridine (4-AP) while molecular presence of Kv on bull spermatozoa by immunoblotting and indirect immunofluorescence. Three sets of 100 µL diluted sperm samples namely- negative control (100 µL of sperm dilution medium (SDM) containing 10×10^6 cells), vehicle control (99 µL of SDM containing 10×10^6 cells, and DMSO- 1µL) and 4-AP treatment group (99 µL of SDM containing 10×10^6 cells, and drug 1 µL 4-AP) were used in the study. Immunoblotting identified a single band of 56 kDa corresponding to Kv1.1 in Hariana bull spermatozoa. Immunolocalization showed the positive immunoreactivity at head, middle piece and principal piece of the spermatozoa for Kv 1.1. Blocking of Kv using 4-AP resulted in significant ($p<0.05$) reduction in sperm progressive motility, per cent capacitated spermatozoa (B-pattern) and acrosome reacted (AR- pattern) spermatozoa, while significant ($P<0.05$) increase in per cent swollen spermatozoa. Spermatozoa treated with 4-AP for 1 h followed by treatment with Pinacidil (1.5mM/100µL SDM/10×10^6 spermatozoa), a non specific opener of potassium channels revived the progressive motility. Blocking of Kv channels resulted in significantly ($P<0.05$) increased percentage of spermatozoa with lower mitochondrial transmembrane potential. Computer assisted semen analysis (CASA) of motion and kinematic parameters in 4-AP treated spermatozoa indicated reduction in sperm motion parameters like LIN, STR, VSL and VAP and higher ALH, VCL, and BCF indicating hyperactivity of spermatozoa. Based on our findings, it may be concluded that voltage-gated potassium channel (Kv) are present on bull spermatozoa and these are associated with