Protein profile and functionality of spermatozoa from two semen collection methods in Bali bulls

Abstract

The aim of this study was to evaluate the effect of two semen collection methods (electro-ejaculation [EE] and transrectal massage [RM]) on in vitro sperm functionality and protein composition of seminal plasma in Bali bulls. Ten untrained Bali bulls were selected for semen collection by EE and RM. Parameters analysed were acrosome and plasma membrane integrity, sperm motility (by CASA), normal morphology, functionality (sperm penetration assay), acrosome reaction, total protein content and protein profiles (by 2D-PAGE). Bulls collected by RM had a higher ($p<0.05$) percentage of spermatozoa with intact acrosome and plasma membrane, functionality and individual motility, and a lower proportion of seminal plasma, total protein content and lower ratio of low molecular weight proteins than those collected by EE. Analysis of 2D-PAGE gel detected about 116 spots in the range of 10–250 kDa and isoelectric points (pI) ranging from 3 to 10. Approximately 52% of seminal plasma protein spots were represented by four major protein fractions with molecular weights around 37–45 kDa (15.66%), 25–30 kDa (12.46%), 14–16 kDa (11.73%) and 12–15 kDa (11.52%). Ten of the seminal plasma proteins identified by mass spectrometry belonged to major bovine seminal plasma proteins. A very significant finding in this study was related to the two proteins identified, PGK and PLA2, with MW of approximately 37–40 and 50–55 kDa and pI of 8.5–8.8 and 5.2–6.0, respectively. These two protein spots can only be detected in the seminal plasma of ejaculates obtained through RM. In conclusion, semen quality as examined by in vitro sperm functionality was found to be better in RM than EE samples after treatment with heparin and calcium ionophore A-23187. In addition some low molecular weight proteins were up-regulated in the seminal plasma obtained from the EE method.

Keyword: Bali bull; Electro-ejaculation; Transrectal massage; Semen protein