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Comparison of protein extraction buffers of bovine sperm

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Protein extraction is the first step to proteomic studies and the success of this technique depends on the association of many factors. One of them is the choice of the best extraction buffer and its interaction mechanism with the cell membrane. For this, we compared three extraction methods for bovine sperm proteins. One ejaculate of a known fertility bull was collected with artificial vagina. Thus, the ejaculate was divided in three samples, centrifuged at 800x for 10 min to remove seminal plasma and recentrifuged at 700x for 10 min for three times in a protease inhibitor buffer. The supernatant was discarded and added to different extraction buffers: 0.1% nonidet P-40 (NP); RIPA and urea/ thiourea/ CHAPS solution (UT). The samples were sonicated (20% amplitude/ 10 x 30s, 60s intervals) and centrifuged at 10000x for 30min. Total protein concentration of supernatant was determined with Pierce 660®, and one-dimensional SDS-PAGE was performed using a 12% separation polyacrylamide gel. Gels were scanned and analyzed through ImageMaster software. Molecular weight of the bands ranged from 11,532 to 365.37 kDA in all groups. Total number bands were 41, 52 and 33 for NP, RIPA and UT, respectively. On RIPA buffer, the protein extraction was higher when compared to other groups ($P<0.05$). IOD of common bands between groups varied and was higher on RIPA group. These results that RIPA is the best buffer to recover sperm bull proteins and have a great application on the 1D gel electrophoresis.

Biography

Caroline Scott has a DVM from University of Franca (UNIFRAN). She obtained her Master's degree in Animal Reproduction from Sao Paulo State University (UNESP/ Botucatu). She is currently a PhD student at the Sao Paulo State University. Her research interest focuses on the proteomics of plasma membrane from bovine sexed sperm.

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