FLOW CYTOMETRY ANALYSIS OF SPERM CHROMATIN STRUCTURE ASSAY AND SPERM VIABILITY ASSAY AND ITS ASSOCIATION WITH BULL FERTILITY

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The objective of the study was to examine the association of flow cytometry analysis of sperm chromatin structure assay (SCSA), sperm viability assay with bull fertility assessed by heterospermic and homospermic indices.

In experiment 1, cows ($N = 422$) were artificially inseminated with frozen-thawed semen containing 20×10^6 spermatozoa/straw of heterosperm, 10×10^6 spermatozoa each from a Jersey and a Holstein bull mixed together. In this study, 4 Jersey and 4 Holstein bulls were used which yielded 16 combinations. After the birth of the calf, DNA genotyping for parentage (Immgen, Inc., College Station, TX 77845) was performed to identify the sire. Heterospermic index (number of calves sired in competition/number of cows exposed) for each bull was estimated to determine bull fertility. In experiment 2, cows ($N = 1124$) were inseminated with frozen–thawed semen using 20×10^6 spermatozoa/straw. In this study, six Holstein bulls were used. Pregnancy diagnosis was performed 40 days post-insemination. Homospermic index (number of cows conceived to a sire/total number of cows inseminated using the sire's semen) was estimated to determine bull fertility. Frozen semen from all bulls ($N = 14$) were thawed and subjected to a flow cytometry analysis to determine SCSA parameters [% DNA fragmentation index (DFI), standard deviation of DFI (SD-DFI)] and sperm viability assay (percentage viable sperm). SCSA parameters were determined using the metachromatic properties of acridine orange to distinguish denatured from intact native DNA. Percentage viable sperm were determined by SYBR-14 and PI staining procedure. Pearson correlation coefficients were performed to estimate the association of sperm chromatin structure assay and sperm viability assay, with heterospermic and homospermic indices.

In experiment 1, the correlations of %DFI, SD-DFI and % viable sperm with heterospermic index were -0.93 ($P < 0.001$), -0.30 ($P > 0.1$) and 0.94 ($P < 0.001$), respectively. In experiment 2, the correlations of %DFI, SD-DFI and % viable sperm with homospermic index were -0.57 ($P = 0.1$), -0.69 ($P = 0.1$) and 0.78 ($P = 0.05$), respectively.

In conclusion, the flow cytometry analysis of SCSA and the sperm viability assay are useful tools in assessing the bull fertility in heterospermic studies.

Keywords: Bull, Spermatozoa; Flow cytometry; SCSA; Sperm viability.