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Effect of bovine sperm chromatin integrity evaluated using three different methods on *in vitro* fertility

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Abstract

In vitro fertility potential of individual bulls is still relatively uncharacterized. Classical sperm analysis does not include the evaluation of all sperm characteristics and thus, some cell compartments could be neglected. In humans, sperm DNA integrity has already proven to have major influence in embryo development and assisted reproduction techniques successfully. In bovine, some studies already correlated chromatin integrity with field fertility. However, none of those have attempted to relate DNA assessment approaches such as chromatin deficiency (CMA3), chromatin stability (SCSA; AO+) and DNA fragmentation (COMET assay) to predict *in vitro* bull fertility. To this purpose, we selected bulls with high and low *in vitro* fertility (n=6/group), based on embryo development rate (blastocyst/cleavage rate). We then performed CMA3, SCSA test and COMET assay to verify if the difference of *in vitro* fertility may be related to DNA alterations evaluated by these assays. For the three tests performed, our results showed only differences in the percentage of cells with chromatin deficiency (CMA3+; high: 0.19±0.03 *vs* low: 0.04±0.04; p=0.03). No difference for chromatin stability and any of COMET assay categories (grade I to grade IV) was observed between high and low *in vitro* fertility bulls. A positive correlation between AO+ cells and grade IV cells was found. Despite the difference between groups in CMA3 analysis, our results suggest that protamine deficiency in bovine spermatozoa may not have a strong biological impact to explain the difference of *in vitro* fertility between the bulls used in this study.