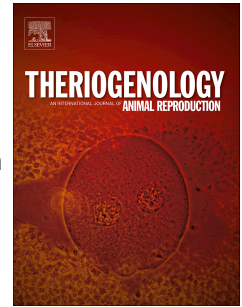


# Accepted Manuscript

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

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PII: S0093-691X(17)30540-X

DOI: [10.1016/j.theriogenology.2017.11.006](https://doi.org/10.1016/j.theriogenology.2017.11.006)

Reference: THE 14338

To appear in: *Theriogenology*

Received Date: 22 May 2017

Revised Date: 3 November 2017

Accepted Date: 3 November 2017

Please cite this article as: Castro LS, Siqueira AFP, Hamilton TRS, Mendes CM, Visintin JA, Assumpção MEOA, Effect of bovine sperm chromatin integrity evaluated using three different methods on *in vitro* fertility, *Theriogenology* (2017), doi: 10.1016/j.theriogenology.2017.11.006.

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13

14 Abstract

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

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