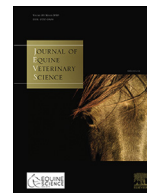




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## Sperm processing methods effectively reduce the DNA fragmentation index in samples with high initial values

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The DNA fragmentation index (DFI) in spermatozoa is correlated to *in vivo* fertility in animals and outcomes in Human Assisted Reproductive Techniques (ARTs). Different sperm selection methods aim to select the most viable sperm population and reduce the DFI after processing. However, it remains unclear which selection method most efficiently reduces DFI and to what extent initial levels in raw semen can be improved to justify the use of these alternative procedures. We hypothesized that sperm selection methods would efficiently reduce the percentage of cells with DNA damage mainly to samples with high initial values. In this study we evaluated frozen-thawed equine spermatozoa from 12 different stallions subjected to four different treatments as follows: i) control / no selection (CN); ii) density gradient centrifugation (DGC); iii) swim up (SU); and iv) a microfluidic chamber (MF). After processing, DFI levels were evaluated based on the percentage of cells outside of the main population (COMP $\alpha$ t) from the Sperm Chromatin Structure Assay (SCSA) as described by Evenson et al., (2013). Levels of COMP $\alpha$ t in the control samples (CN) were used to categorize them as LOW and HIGH based on a median split (Mdn:12.37%). Statistical analysis was performed using R (R Core Team, Vienna, Austria, v3.6.0) to fit a mixed linear regression

model. Fixed effects in the model included treatment (CN, DGC, SU and MF) and initial DFI levels (LOW or HIGH). Random effect in the model included stallion (n=12) and effects were retained in the model when  $p < 0.05$ . There was a significant interaction between treatment and initial DNA fragmentation levels ( $p < 0.00001$ ). In samples with HIGH DFI, COMP $\alpha$ t had a mean of (20.44%  $\pm$  1.68) for CN and was not significantly different from samples processed by DGC (14.36%  $\pm$  1.68;  $p = 0.07$ ). However, a significant reduction in COMP $\alpha$ t was observed after SU (2.97%  $\pm$  1.68;  $p < 0.0001$ ) and MF (1.95%  $\pm$  1.68;  $p < 0.0001$ ) in this group. On the other hand, among samples in the LOW DFI group, COMP $\alpha$ t had a mean of (8.32%  $\pm$  1.68) for CN, which was not significantly different when compared to DGC (7.34%  $\pm$  1.68;  $p = 0.99$ ), SU (3.24%  $\pm$  1.68;  $p = 0.2$ ) or MF (2.07%  $\pm$  1.68;  $p = 0.059$ ) selection methods. These results demonstrate that SU and MF were the most effective at reducing the percentage of cells with DNA fragmentation (COMP $\alpha$ t) when the initial DFI was considered HIGH. However, in samples with LOW DFI, there was no significant improvement in COMP $\alpha$ t by any selection method. Clinical categorization of initial sperm DFI may be warranted to determine the fecundity of sires and be useful for selecting appropriate sperm selection methods for equine ARTs.