Relationships between sperm chromatin structure, DNA breakage of sperm and seasonal pregnancy rate of stallions

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While in the last few years the sperm chromatin structure assay (SCSA®) was mainly applied to determine the DNA integrity of equine sperm, in a recent study the single cell gel electrophoresis assay (COMET) was used for the first time to detect DNA breakage in equine spermatozoa. As the COMET assay is not such expensive as the SCSA®, the aim of this study was to investigate the relationships between the results of both tests to fertility data in stallions.

From each of 30 stallions one ejaculate was collected and cryopreserved. All investigations were carried out immediately after thawing. The SCSA® is based on the susceptibility of sperm chromatin to acid-induced denaturation in situ. Using the metachromatic dye acridine orange the percentage of single (red fluorescence) and double stranded DNA (green flurescence) on a single cell level can be measured by flow cytometry. The extent of denaturation was quantified using the mean value of DFI (defragmentationindex) of 5000 sperm cells (DFI = red/(red+green) fluorescence per cell). The COMET, which was used for detection of DNA strand breakage, measures DNA fragmentation in individual sperm nuclei based upon a gel electrophoretic pattern. Broken DNA strands migrate towards the anode and form a comet tail – the larger the tail, the greater the extent of damage. The size of tail was quantified by a computerised image analysis system using the parameter tail moment. The tail moment is the integrated value of density multiplied by migration distance. The fertility estimate for each stallion was based on its seasonal pragnancy rate (SPR).

The tail moment was 5.5$\pm$1.6 (mean ± SD) and mean of DFI was 218.0$\pm$26.1 (mean ± SD). Between mean value of DFI and tail moment a weak positive relationship ($r = 0.42$; $p < 0.05$) occurred. While mean values of DFI were negatively related to SPR ($r = -0.60$; $p < 0.01$), no significant correlation between the tail moment and SPR could be observed ($r = -0.25$;.$p > 0.05$). The results show that there is a weak dependency between alterations in sperm chromatin structure and DNA fragmentation, but only the changes in DNA integrity measured by SCSA® seem to be related to fertility of stallions.

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