may have positive effects on spermatozoa motility after 48 h cooling, and further investigation is warranted.

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Equine spermatozoa viability comparing the NucleoCounter SP-100 and the eosin–nigrosin stain

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The objective of this study was to assess spermatozoal viability using eosin–nigrosin stain (E–N) and the NucleoCounter SP-100 (NC) and correlate viability to progressive motility and morphology. Spermatozoal viability or integrity of the sperm plasma membrane can be evaluated in several ways. Under field conditions, spermatozoal viability can be assessed using either classical stains, like E–N, or using the NC. When using the E–N stain, eosin is occluded from spermatozoa with an intact membrane, but penetrates membrane damaged cells. E–N is a subjective test using light microscopy and 100–200 spermatozoa are counted. The NC is an integrated fluorescence microscope that counts the number of spermatozoa (approximately 1500) stained with propidium iodide (PI). PI has been used extensively to stain non-viable spermatozoa (Love et al., 2003). Looking at boar semen the accuracy and repeatability of the NC was identical to the flow cytometer (Hansen et al., 2006). Further information on the NC at www.chemometec.dk. Spermatozoa viability using E–N and the NC as well as progressive motility and morphology were assessed in 72 ejaculates (8 ejaculates from 9 stallions) on a Danish stud farm at regular intervals throughout the breeding season 2007. Of the evaluated ejaculates, 3 were not included in the dataset due to suboptimal E–N slide preparation. Progressive motility was assessed immediately after collection in extended semen. Spermatozoal morphology was determined using the E–N stain (200 cells). In 67 of the 69 evaluated ejaculates the percentage of viable cells was higher when determined by E–N compared to the NC. The mean difference in spermatozoal viability determined by E–N and NC was 12.5%, but the difference was not significant ($p = 0.09$, two sample test for proportions). Spearman's correlation coefficient (r) was 0.64 ($p < 0.001$). Sperm viability determined by E–N and NC correlated significantly to progressive motility and morphology: $r = 0.72$ and 0.57 and $r = 0.79$ and 0.60 ($p < 0.001$), respectively. The fact that viability in 67 of 69 ejaculates was higher when using E–N compared to the NC, could be explained by the fundamental difference in the two methods (eosin vs. PI). The subjectivity of E–N could very likely be a cause of variation since the difference between eosin stained or unstained spermatozoa is sometimes subtle.

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Prostaglandin E_2 concentrations and sperm motility in fractionated stallion ejaculates

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Stallion ejaculates are formed by several fractions that differ in their composition and sperm concentration. Information on prostaglandin levels in stallion seminal plasma (SP) is scarce, but it has been shown that decreased levels of prostaglandin E (PGE) associated with NSAID therapy do not affect semen characteristics and freezability (Janett et al., 2005). The purpose of this study was to compare the concentrations of PGE_2 in different fractions of SP, and to evaluate the correlation of the concentration and total amount of PGE_2 with semen volume, sperm concentration and motility. Semen from 41 stallions was collected as three separate fractions: the first sperm-rich fraction with possible pre-ejaculatory fluid (FR1), the latter portion of the sperm-rich fraction (FR2) and the sperm-poor fraction (FR3). The volume of each sample was recorded and the sperm concentration was determined with a Bürker counting chamber. A portion of each fraction was combined to a sample representing the whole ejaculate (WE). A part of each fraction was centrifuged at $4000 \times g \times 15$ min, then the supernatant was removed, filtered with 0.45 μm filters and frozen (−75 °C) in 1.5-ml vials. The remaining part of each fraction was centrifuged at $500 \times g \times 10$ min, resuspended in a mixture of SP and skim milk extender (ratio 1:2) and stored at 5 °C for 24 h. The PGE_2 concentrations in SP were analyzed with commercial enzyme immunoassay kit (Assay Designs Inc., MI, USA). A computerized motility analyzer (SpermVision) was used to determine sperm motility parameters. There were significant differences between fractions in the concentration of PGE_2. FR3 had significantly lower values (257.0 ± 92 ng/ml) compared with the other fractions (FR1: 359.4 ± 190 ng/ml, FR2: 387.7 ± 167 ng/ml and WE: 353.5 ± 134 ng/ml). No significant correlations were found between the concentration or total amount of PGE_2 and sperm concentration, total sperm count or semen volume, even though the sperm concentration was significantly lower in FR3 (123×10^6 sperm/ml) compared with FR1 (284×10^6 sperm/ml) and FR2 (240×10^6 sperm/ml). The differences between fractions in total motility and progressive motility were non-significant, but the sperm average path velocity was higher in FR3 compared with FR1, FR2 and WE. There were no significant correlations between PGE_2 levels and the motility parameters. The lowest PGE_2 concentration was found in the last sperm-poor fraction of the ejaculate. PGE_2 levels were not correlated to any of the measured