

## The use of iSperm technology for on-farm measurement of equine sperm motility and concentration

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The iSperm® is a newly released semen analysis tool from Aidmics Biotechnology CO. LTD, which allows an ordinary iPad mini to be transformed into a hand-held microscope with objective semen analysis software for equine available through the Apple Store (version 4.5.2). The aim of this study was to compare iSperm® values for sperm motility and sperm concentration to current acceptable methods for semen analysis and to determine the agreement with these methods using statistical methods. Two ejaculates from each of five Standardbred stallions were used in this study to compare sperm motility and concentration. For motility analysis, spermatozoa were diluted to approximately  $30 \times 10^6/\text{ml}$  in INRA 96. An aliquot was then snap-frozen in liquid nitrogen and used to prepare samples with varying motilities by systematically adding a percentage of dead spermatozoa to the fresh sample (live:dead; 100:0, 75:25, 50:50, 25:75). Each sample was measured in triplicate by CASA (Ceros, Hamilton Thorne, Inc.) and iSperm®. Endpoints included total motility (TM), progressive motility (PM) and average path velocity (VAP). For concentration determination, samples were centrifuged at  $500 \times g$  for 10 minutes and resuspended to approximately  $480 \times 10^6/\text{ml}$ . A serial dilution was then prepared to achieve concentrations of 240, 120, 60 and  $30 \times 10^6/\text{ml}$ , which spans the reported accuracy interval for iSperm® (20 to 500 mil/ml). Samples were evaluated in triplicates with both the iSperm® and Nucleocounter SP-100 (ChemoMetec A/S). Data were analyzed by first testing for the differences between the means of each method using a linear mixed model in R statistical software. The model accounted for the effect of stallion, ejaculate within stallion and the method of measurement. The 'lme4' and 'lmerTest' R-packages were used for model fitting and hypothesis testing. The agreement between the two continuous measurements for each method was then investigated by computing Lin's concordance correlation coefficient in the 'epiR' R-package. This measurement combines measures of both precision and accuracy to determine how close the data are to the line of perfect concordance with values ranging from 0 (no agreement) to 1 (perfect agreement). Results are reported as the Lin's coefficient with the associated 95% confidence interval in parentheses. Means for both TM and VAP were different between CASA and iSperm® readings ( $P < 0.001$ ). However, PM means were not different between CASA and iSperm® values ( $P = 0.852$ ). For concentration, means were not different between Nucleocounter and iSperm® values for the five concentrations analyzed ( $P = 0.578$ ). The Lin's concordance correlation coefficient for TM was 0.831 (0.774, 0.875). For PM, the correlation coefficient was 0.883 (0.836, 0.917). Average path velocity had a much lower correlation coefficient of 0.072 (0.043, 0.101). Finally, the correlation coefficient for concentration compared by iSperm® and Nucleocounter was 0.953 (0.936, 0.966). With values close to the line of perfect concordance for TM, PM and concentration it is concluded that there is good repeatability between the two sets of data for each method used. Since the software is still undergoing development, it is recommend that users maintain the software updated with the latest developments. The iSperm® will introduce a low cost and affordable method for on farm semen analysis for breeders and veterinarians. As a result, more farms will have access to accurate sperm analysis tools which will help to standardize semen processing procedures leading to better overall quality of semen used for artificial insemination.

**Keywords:** Spermatozoa, motility, equine, iSperm®, CASA