Morphological evaluation of the stallion spermatozoa through three staining methods
E. Jouanisson, Y. Murcia, G. Beauchamp, M. Diaw
Department of Clinical Sciences, Faculty of Veterinary Medicine, Université de Montréal, Saint-Hyacinthe, Quebec, Canada

Evaluation of sperm morphology is part of the assessment of fertility in human or animal reproduction. The analysis can be performed using different techniques, including the use of staining methods. In our prospective study, morphology of the sperm of the horse was evaluated using three staining methods: Diff Quik® (Siemens Healthcare Diagnostics Inc., Deerfield, IL), Eosin-Nigrosin® (RAL Diagnostics, Martillac, FR) and Spermblue® (Microptic Automatic Diagnostic Systems, Barcelona, SP), a stain used for human sperm. Our hypotheses were that (1) Spermblue® would allow an easier reading of the sperm morphology of the horse and allow a better identification of sperm abnormalities, (2) there would be differences in classification of sperm morphology depending on the evaluator's experience. Semen was obtained from various breeds of horses. Forty samples from stallions between 2 and 15 years of age were collected during the 2016 breeding season and stored in a 2% buffered formaldehyde solution until processed. For each sample, three semen smears were made and stained with Diff-Quik®, Eosin-Nigrosin® and Spermblue®. All morphological parameters were then evaluated blindly using a light microscope by a novice evaluator and a more experienced evaluator. For each slide, 200 spermatozoa were examined randomly and were classified into eleven categories according to morphology: normal, abnormal acrosome, abnormal head, detached head, abnormal midpiece, bent midpiece, proximal droplets, distal droplets, abnormal tail, bent tail, round cells and others. Statistical analysis was performed using linear mixed model; staining and observer were within-subject factors. There were no replicate experiments. Independently of the staining methods used, there was no significant difference between evaluators in the identification of morphologically normal spermatozoa. In contrast, significant differences between evaluators were observed in the classification of some anomalies affecting mainly the midpiece and the tail. A poor fixation of the dye was also observed with Spermblue®.

Keywords: Stallion; sperm morphology; staining methods; Spermblue