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Implementing an open-access CASA software for the assessment of stallion sperm motility: Relationship with other sperm quality parameters

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ABSTRACT

Setting an open-access computer assisted sperm analysis (CASA) may benefit the evaluation of motility in mammalian sperm, especially when economic constraints do not allow the use of a commercial system. There have been successful attempts to develop such a device in Zebra fish sperm and the system has been used in very few studies on mammalian spermatozoa. Against this background, the present study aimed at developing an open-access CASA system for mammalian sperm using the horse as a model and based upon the Image J software previously established for Zebra fish sperm. Along with determining the sperm progressive motility and other kinetic parameters (such as amplitude of lateral head displacement), the "results" window was adjusted to simplify subsequent statistical analyses. The path window was enriched with colored sperm trajectories on the basis of the subpopulation they belong to and a number that allowed the sperm track to be associated to the sperm motility data shown in the "results" window. Data obtained from the novel plugin (named as CASA_bgm) were compared with those of the commercial CASA Hamilton-Thorn IVOS Vers.12, through Bland Altman's plots. While the percentage of total and progressive motile sperm, VCL, VAP, VSL, LIN and STR and ALH were in agreement with those obtained with the commercial system, BCF significantly differed between the two systems probably due to their settings. Interestingly, a positive and significant correlation between the percentages of total motile sperm evaluated through CASA_bgm and those showing high mitochondrial membrane potential evaluated by JC-1 staining was found. In conclusion, CASA_bgm ImageJ plugin could be useful and reliable for stallion sperm motility analysis and it is our aim to apply this system to other mammalian species.

1. Introduction

At present, more than 12 different computer-assisted sperm analysis (CASA) systems are available for sperm motion detection in spermatology labs and in commercial semen production units (Amann and Waberski, 2014). The development of a powerful CASA software has made possible kinetic studies of spermatozoa and objective measurements of sperm movements (Verstegen et al., 2002).

The evaluation of sperm motility and other kinetic parameters such as curvilinear, straight line, and average path velocities, is an essential part of sperm quality

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examination in many mammalian species. Despite the immediacy and accuracy of these softwares, several investigators rely on non-automated analysis due to the high cost of commercial options. CASA systems historically evolved for commercial purposes and were initially sold to clinical laboratories to assess human sperm fertility (Amann and Katz, 2004). Later on, CASA systems were produced for stallion sperm analysis by Hamilton-Thorne in 1986, and soon after they were adapted to many species. In spite of being much widespread across research laboratories, "teaching the instrument" is still needed, as reported by Amann and Katz (2004).

The availability of an open-access, low cost CASA system could much benefit the analyses of sperm motility, especially for those that, due to economic constraints, may not afford the costs of a commercial device. In addition, the relevant information that a CASA system provides goes beyond a more objective evaluation of the percentages of total and progressive motile sperm. Indeed, some CASA systems give the individual kinetic parameters for a single sperm cell and this may be used for evaluating motile sperm population in differently treated samples (Abaigar et al., 1999; Flores et al., 2009, 2008; Miró et al., 2005, 2009; Schmidt and Kamp, 2004; Varner, 2008). In 2007, Wilson-Leedy and Ingermann developed a CASA software package that worked as a plugin to the United States National Institutes of Health (NIH) Image J software (Wilson-Leedy and Ingermann, 2007). Successively, more than hundred scientific studies used the plugin to assess fish or invertebrate sperm motility. In particular, Purchase and Earle (2012) implemented the original plugin, creating a new one that permits the automation of some video processing steps. Very few studies applied the system to mammalian sperm motility evaluation. Elsayed et al. (2015) used the plugin to study sperm motility in bull and adapted the system to their specific experimental conditions. Boryshpolets et al. (2015) used the original plugin to study human sperm motility in response to thermotaxis. Since this plugin deposited is an open source, this allows any research laboratory to have access to CASA software and to perform the motility sperm analysis.

The first aim of our study was to set up an Image J CASA system for mammalian sperm analysis, using the horse as a model, and also including progressive motility and amplitude of lateral head displacement measurements; second, we compared the results obtained with our system to those of a commercial one and to the data reported in the literature. Finally, we checked the correlations between motion values obtained from the two CASA systems and other parameters of semen quality such as mitochondrial activity and acrosome integrity.

2. Materials and methods

2.1. Collection and preparation of semen

Twenty-five ejaculates were collected from four Standardbred stallions of proven fertility, individually housed at the National Institute of Artificial Insemination (University of Bologna, Italy), using a Missouri artificial vagina with an inline filter (Nasco, Fort Atkinson, WI, USA). Semen was diluted in Kenney's extender (Kenney et al., 1975) at a semen/extender ratio of 1:3 (v:v) and sent to the laboratory within 1 h post-collection at 20–25 °C.

Upon arrival, an aliquot of 2 mL of extended semen was further diluted to a final concentration of 30×10^6 spermatozoa/mL, and then split into three aliquots. One was evaluated with the Hamilton-Thorne CASA system Vers.12, another was evaluated with the Image J software and the new CASA_bgm, and the last one was used to evaluate the sperm viability with mitochondrial membrane potential and acrosome integrity.

2.2. Video microscopy system for motility assessment

Settings for video camera and microscope were established according to the indications of Wilson-Leedy and Ingermann (2007) and a Leitz diaplan microscope (Wild Leitz GmbH, D6330, Wetzlar, Germany) with a 10x plan objective with negative phase-contrast was used. The microscope was equipped with a Z31A Ascon technologic heated stage (Ascon technologic, PV – IT). The video camera, 3.1 megapixel CMOS USB 2.0 Infinity1-3 Camera (Lumenera corporation, Ottawa, ON, Canada), was coupled to the microscope by a c-mount adapter and videos were registered for three seconds at a resolution of 800×600 pixel and 60 frames/s (fps). Images were recorded on a hard drive using the Infinity analyzing and capture software 6.4 (Lumenera corporation) and converted to avi format.

Prior to any observation, spermatozoa (30×10^6 sperm/mL) were loaded onto a fixed height Leja Chamber SC 20-01-04-B (Leja, CIUDAD; The Netherlands). Five videos of separate fields and lasting three seconds each were recorded per sperm sample.

2.3. Installation of plugin and video adjustment and analysis

The indications of Wilson-Leedy and Ingermann (2007) were followed to install the plugin and to import the central second of each video. After importing, each video was converted into greyscale 8-bit image and the threshold was adjusted to highlight the sperm heads over the background.

Launching the plugin results in the initiation of a dialog box, where parameters for analysis need to be indicated. In order to adapt the existing plugin to the analysis of mammalian spermatozoa, the input parameters related to the bulk flow were eliminated. Therefore, the dialog box generated for CASA_bgm is more simple than that of CASA and the implemented input settings consisted of two VAP cut-off values that divided the sperm population into slow, medium and rapid spermatozoa. The input parameters used to identify and characterize the sperm motion are reported in Fig. 1.

As shown in Fig. 1a, the first two (a and b) parameters regard the minimum and maximum pixel areas that the program takes into account for the analysis. The particles over or below the selected area are not analyzed. The minimum track length (c) indicates the minimum number of frames in which a particle must appear in the video in order to be considered in the analysis. The maximum sperm velocity between frames (d) regards the maximum distance