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# Evaluation of a portable device for assessment of motility in stallion semen

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## Contents

In horse breeding, quality assessment of semen before insemination is often requested. Non-laboratory-based techniques for objective analysis of sperm motility are thus of interest. The aim of this study was evaluating a portable device for semen analysis (Ongo sperm test) and its comparison with computer-assisted semen analysis (CASA). Semen was collected from 10 stallions, diluted to 100, 50 and $25 \times 10^6$ sperm/ml and analysed for total (TM) and progressive motility (PM). The final sperm concentration influenced total motility analysed by Ongo ($p < 0.05$) which was higher at $100 \times 10^6$ sperm/ml when compared to $25 \times 10^6$ sperm/ml ($p < 0.05$) but not when compared to $50 \times 10^6$ sperm/ml (n.s.). Sperm concentration did not influence total motility when assessed by SpermVision (n.s.). Agreement between methods was evaluated by correlation analysis and Bland–Altman plot. Intra-assay variation of Ongo was 5.2% ± 3.0 for TM and 6.9% ± 3.4 for PM. Correlation between Ongo and CASA was $r = 0.79$, 0.88 and 0.83 for 100, 50 and $25 \times 10^6$ sperm/ml for TM, and $r = 0.87$, 0.89 and 0.87 for PM, respectively (all $p < 0.001$). At the 100 and 25 mio/ml dilutions, the difference between the two systems deviated significantly from 0, while no such bias existed at the 50 mio/ml dilution (TM Ongo 85.0%, CASA 82.3%; PM Ongo 64.1%, CASA 66.1%). The 95% confidence interval was 19.9%, 18.9% and 19.2% ± mean for TM and 20.7%, 17.4% and 20.3% ± mean for 100, 50 and $25 \times 10^6$ sperm/ml, respectively. In conclusion, Ongo sperm test sperm motility data were strongly correlated with data obtained by CASA. In addition, at a concentration of $50 \times 10^6$ sperm/ml values measured with both systems were close to identical. At this concentration, which is recommended in equine AI, Ongo and CASA can be used interchangeably.

**KEYWORDS**

computer-assisted semen analysis, semen motility, stable test

## 1  |  INTRODUCTION

For decades, semen motility has been assessed by estimation under a phase contrast microscope. The development of computer-assisted semen analysis (CASA) approximately 40 years ago (Jasko, Little, Smith, Leinl, & Foote, 1988) and the steadily increasing number of publications in this field (Verstegen, Iguer-Ouada, & Onclin, 2002) have allowed for a more accurate and reliable determination of semen motility (reviewed by Amann & Waberski, 2014).

The evolution of CASA has seen innovation, leading to more detailed information on morphology and characteristics of sperm cell motility (Love, 2011; Mortimer, Horst, & Mortimer, 2015). The positive impact of CASA on sperm quality control in breeding stallions is well documented (Jasko, Lein, & Foote, 1991; Loomis, 2006; Loomis & Graham, 2008; Love, 2011). The use of commercially available CASA systems, however, has been largely restricted to a laboratory setting because of the high costs, and hardware requirements of such systems. A microscope connected to a computer is inherently unsuited to field use. The benefits of a portable field-use device that would increase the range of CASA applications in veterinary medicine as well as in human assisted reproduction have been discussed (Amann & Waberski, 2014). Evaluation of semen from EU-approved collection centres has shown a wide variation in semen characteristics due to processing and storage conditions of cooled-shipped semen (Heckenbichler, Deichsel, Peters, & Aurich, 2011). The availability of a portable device for CASA motility analysis might help to improve the assessment of characteristics of raw semen and semen during processing in semen collection centres for stallions that in contrast to large bull AI centres will usually not afford the purchase of sophisticated CASA machines. As semen characteristics may decrease with less than optimal handling (Brinsko, Rowan, Varner, & Blanchard, 2000; Heckenbichler et al., 2011; Varner, Blanchard, Love, Garcia, & Kenney, 1987a), the need for quality control at the time of insemination is also given and the availability of a field-use device would be appreciated by many veterinarians.

Measuring the accuracy and repeatability of a CASA motility system depends on the application of standardized equipment, operating procedures and adequate training of technicians (reviewed by Yeste, Bonet, Rodríguez-Gil, & Rivera Del Álamo, 2018). Internal image settings such as minimum contrast, frame rate (number of images per second) or analysis time are important for identification and reconstruction of the trajectory of the spermatozoa and clearly affect CASA results (Rijsselaere, Soon, Maes, & Kruif, 2003). Improvement of hardware and software today allows for the routine use of frame rates between 30 and 60 Hz. For reliable analysis of mammalian sperm motion characteristics, >50 frames per second are recommended (Mortimer, 2000); however, spermatozoa are less likely to stay undetected with lower frame rates (Yeste et al., 2018). Conflicting results with regard to velocity parameters may depend on different frame rates even when the same CASA system is used. Increasing frame rates result in increasing velocity parameters with curvilinear velocity (VCL) being most sensitive and straight line velocity (VSL) being least sensitive to this influence (Bompart et al., 2018). Additionally, also type and features of the counting chamber will impact on results of CASA motility analysis (Hoogewijs et al., 2012). Standardization of systems and settings, however, still does not guarantee comparability among different systems (Amann & Waberski, 2014; Yeste et al., 2018). Validation needs to be specific with regard to the system, its settings and the species investigated (Verstegen et al., 2002).

In this study, a portable device for assessing semen motility was compared to a laboratory-based CASA system. The aim was to investigate the reliability and repeatability of measurements obtained with the portable device, the ability to obtain consistent results when measuring at different semen concentrations, as well as the agreement of results with those obtained by CASA. We hypothesized that satisfying results can be obtained with the portable system if semen concentration is optimized for analysis.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

A total of 10 stallions of proven fertility from different breeds were included into the study (two Warmbloods and eight Shetland ponies). Semen from Shetland ponies was collected within a study approved by the Austrian Federal Ministry for Science and Research (licence number BMWFW-68.205/0150-WF/V/3b/2015). The warmblood stallions were approved sires, and semen was collected for commercial semen shipment in accordance with EU Directive 92/65/EEC. Age of the stallions was 7.5–25.9 years (15.3 ± 7.4). Warmblood stallions were housed in individual loose boxes on straw and had access to an outdoor paddock for 1–2 hr per day. They were fed oats and concentrates three times daily and hay twice daily. Water was available at all times. Shetland stallions were housed in a group in an outdoor paddock at all times and had access to a covered shed. They were fed hay twice daily and water was always available.

### 2.2 | Experimental design

For this study, a portable device for assessing semen motility (Ongo Sperm Test®, Microfluidlabs, Budapest, Hungary) was compared to a laboratory CASA system (SpermVision, Minitube, Tiefenbach, Germany). In the first trial, three different sperm dilutions (100, 50 and 25 × 10^6 spermatozoa/ml) were analysed with both systems. In the second trial, five repeated measurements with the Ongo system using a sperm dilution of 25 × 10^6 sperm/ml were made.

### 2.3 | Experimental procedures

#### 2.3.1 | Semen collection

Semen was always collected immediately before testing procedures with a Hannover model artificial vagina (Minitube) on a dummy. For semen collection, stallions were exposed to a teaser mare until erection and readiness to mount, followed by mounting of the dummy. Immediately after semen collection, the gel fraction of the ejaculate was removed. Semen was filtered through sterile gauze before further analysis and ejaculate volume, colour and consistency were determined. The pH was determined with test strips (Merck, Darmstadt, Germany).

#### 2.3.2 | Semen analysis

Sperm concentration was measured with a Nucleocounter SP-100® (Chemometec, Allerød, Denmark) as described (Comerford et al.,

2008). Total sperm count was calculated from ejaculate volume and sperm concentration.

In all ejaculates, the percentage of motile and progressively motile spermatozoa was evaluated with the Ongo and SpermVision systems. Raw semen was diluted with EquiPlus extender (Minitube) to final concentrations of 100, 50 and $25 \times 10^6$ sperm/ml. Higher concentrations were measured before lower concentrations in order to minimize degradation over time, and corresponding measurements were carried out with SpermVision and Ongo in immediate succession to ensure comparable quality of the samples.

Ongo sperm test is a portable device for automated assessment of semen motility. It consists of an optical system, a screen, a warmed stage for samples (37°C), menu buttons, a processor, a battery and a USB interface. A sample is pipetted into a chamber on a slide. The sample is illuminated by a green LED integrated into a lid which closes on the sample from above. The optical system transmits the magnified sample to the screen, where it can be focused and motility judged subjectively, if desired. The on-screen magnification is approximately 250×. Analysis is started by pressing a start button, whereupon a 2-s video is recorded at 30 frames per second (fps). The measurement can be repeated until a pre-determined number of cells have been analysed. The increasing total cell count as well as weighted averages of motility results is displayed after each new video analysis. Measurement results are stored internally in a log file, and all videos are stored in.avi format. These files can be downloaded via a USB interface to a computer for viewing and for documentation purposes. In this study, Ongo measurements were carried out until at least 1,000 cells had been analysed. Therefore, the number of videos recorded to reach this goal varied depending on the sample concentration. Ongo tracks sperm cells, analyses their motility parameters and categorizes them according to WHO categories (WHO, 2010). This includes four grades: grade a is defined as rapid progressive motility (≥25 μm/s), grade b as slow or sluggish progressive motility (5–25 μm/s; sluggish means slow or rapid motion with circular, non-linear direction), grade c as non-progressive motility (<5 μm/s) and grade d as immotility.

The on-screen output after analysis consists of the concentration and the percentages of total motility (grades a, b and c), progressive motility (grades a and b), local motility (grade c) and immotile spermatozoa (grade d).

With the SpermVision CASA system, the percentage of motile, progressively motile and membrane-intact spermatozoa was evaluated as described (Heckenbichler et al., 2011; Pagl, Aurich, Müller-Schlösser, & Aurich, 2006; Schäfer-Somi & Aurich, 2007). Thirty frames per field were evaluated. To select cells from debris, the camera recognizes the position of the sperm heads in successive frames. At least eight fields per sample with approximately 100 cells per field were evaluated. Spermatozoa with average orientation change <8 μm were considered immotile. Spermatozoa with curvilinear velocity ≥10 μm/s, distance straight line ≥6 μm and radius ≥15 μm were considered progressively motile. SpermVision categorizes sperm cells as progressively motile, locally motile and non-motile. Total motility is thus progressive plus local motility.

SpermVision measurements were carried out until at least 700 cells were measured. Samples were analysed in slides produced for use with SpermVision (Minitube). There are four chambers per slide, each 20 microns deep and laid out for a sample volume of 3 μl. Slides were warmed to 37°C before loading and placed on a warmed stage for analysis.

## 2.4 | Statistical analysis

Because not all data were normally distributed (Kolmogorov–Smirnov test), motility data (total and progressive motility) assessed with either Ongo or SpermVision at different sperm concentrations (25, 50 and $100 \times 10^6$ sperm/ml) were compared by Kruskal–Wallis test with subsequent Mann–Whitney test. Motility data are presented as mean ± *SEM* (standard error of mean). The correlation between motility data obtained by Ongo and SpermVision CASA was analysed by calculating Pearson's coefficient of correlation. Agreement between data obtained with the two CASA systems was also assessed by Bland–Altman plot (Bland & Altman, 1986). The differences between data from both systems were plotted against their mean. Confidence intervals of the mean were calculated. Upper and lower limits of agreement were calculated as the mean of the differences ±1.96 *SD* of the differences. Limits of agreement of <20% were deemed to be acceptable for using both methods interchangeably in a clinical setting (Mortimer et al., 2015). Some data sets were not normally distributed due to the relatively low number of samples. Therefore, bootstrapping was employed to calculate 95% confidence intervals and *SD*. This method differs from parametric approaches in that it can yield valid results from data that is not distributed normally. The intra-assay variation was evaluated by calculating the coefficient of variation. All statistical analyses were made with the spss software (version 21.0; IBM-SPSS, Armonck, NY, USA). A *p*-value < 0.05 was considered statistically significant.

## 3 | RESULTS

### 3.1 | Motility analysis with Ongo and SpermVision

The final sperm concentration influenced total motility analysed by Ongo ($p < 0.05$) which was higher at $100 \times 10^6$ sperm/ml when compared to $25 \times 10^6$ sperm/ml ($p < 0.05$) but not when compared to $50 \times 10^6$ sperm/ml (n.s.). Sperm concentration did not influence total motility when assessed by SpermVision (n.s.). In contrast, analysis of progressive motility by SpermVision resulted in lower values at $100 \times 10^6$ sperm/ml when compared to $25 \times 10^6$ sperm/ml ($p < 0.05$) but not when compared to $50 \times 10^6$ sperm/ml (n.s.). Progressive motility was not influenced by sperm concentration when analysed by Ongo (Table 1).

### 3.2 | Correlation between Ongo and SpermVision

Pearson's coefficient of correlation between Ongo and SpermVision data was highest for the $50 \times 10^6$ sperm/ml concentration ($r = 0.88$), followed by 25 ($r = 0.83$) and $100 \times 10^6$ sperm/ml ($r = 0.79$) for

**TABLE 1** Total motility (%; mean ± *SEM*) and progressive motility (%; mean ± *SEM*) in equine semen at final concentrations of 25, 50 and 100 × $10^6$ sperm/ml (*n* = 10 per concentration) after analysis with either the Ongo or the SpermVision CASA system

| Sperm concentration (×$10^6$ sperm/ml) | Total motility (%) | | Progressive motility (%) | |
|---|---|---|---|---|
| | Ongo | SpermVision | Ongo | SpermVision |
| 25 | 76.7 ± 5.3[a] | 86.9 ± 3.0 | 62.7 ± 6.0 | 75.5 ± 4.3[a] |
| 50 | 85.0 ± 5.2[ab] | 82.3 ± 3.3 | 64.1 ± 6.3 | 66.1 ± 4.6[bc] |
| 100 | 90.3 ± 4.8[b] | 74.1 ± 5.3 | 64.8 ± 6.6 | 48.0 ± 6.0[c] |

*Note.* abc: values with different superscripts within the same column differ significantly (*p* < 0.05).
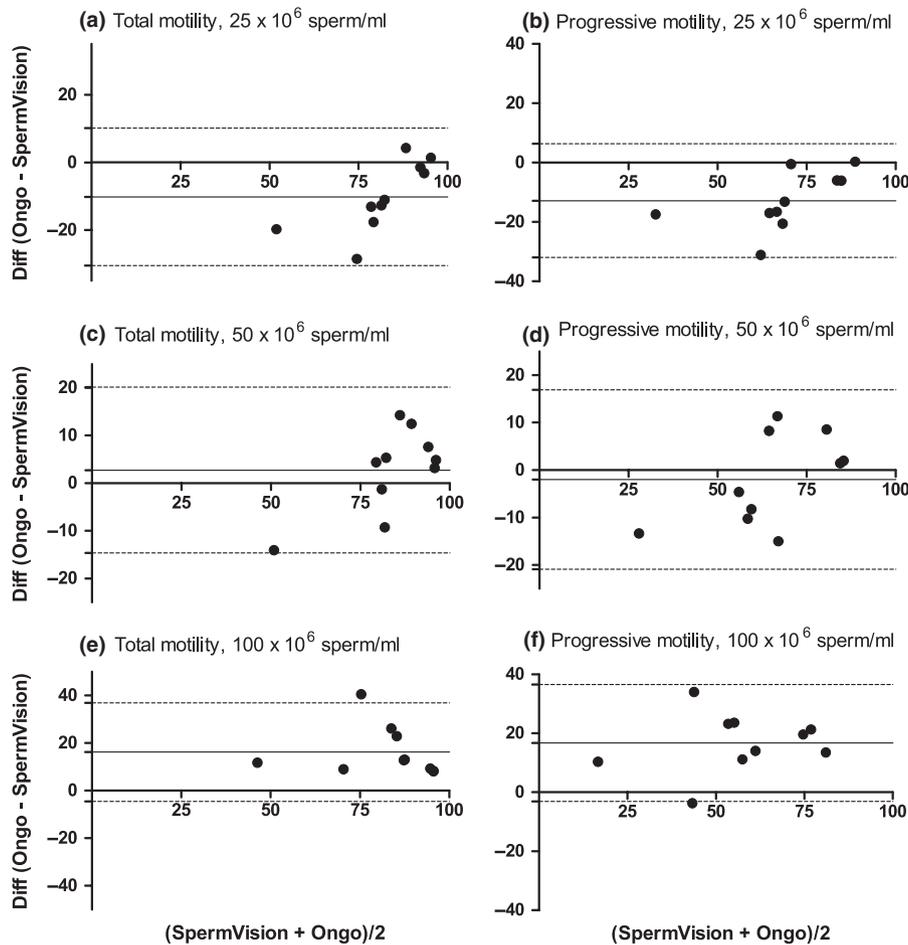


**FIGURE 1** Bland–Altman plots of total and progressive motility at 25, 50 and 100 × $10^6$ equine sperm/ml (*n* = 10 samples per concentration). The thin solid line is the average of the differences, the dotted lines are the 95% upper and lower limits of agreement. All values are given as percentages

total motility (all *p* < 0.01). For progressive motility, the correlation at 25 × $10^6$ sperm/ml was *r* = 0.89, and for both 25 and 100 × $10^6$ sperm/ml *r* = 0.87 (all *p* < 0.001).

## 3.3 | Agreement between Ongo and CASA

Bland–Altman charts for 100, 50 and 25 × $10^6$ sperm/ml were prepared. Best agreement was found at 50 × $10^6$ sperm/ml, and there was no significant bias at this concentration. Mean of the differences (Ongo—SpermVision) was −1.98 and 2.75 for progressive and total motility, respectively, with 95% confidence intervals of ±5.97 and 5.50. Both confidence intervals include the *x*-axis. Limits of agreement

were mean ± 15.57 and 16.88 for progressive and total motility, respectively. At 100 × $10^6$ sperm/ml, there was a positive difference between the results from Ongo and SpermVision (16.74 for progressive and 16.17 for total motility), whereas at 25 × $10^6$ sperm/ml there was a negative difference (12.80 for progressive and 10.17 for total motility). The *x*-axis was outside of the confidence intervals at these concentrations. Limits of agreement were all <20% ± mean (Figure 1a-f).

## 3.4 | Intra-assay variation of Ongo

Coefficients of variation (CoV) were calculated for five repeated measurements of each of the 10 samples at 25 × $10^6$ sperm/ml. For

total motility, the range of all CoV was 1.58%–9.72%, with a mean of 5.2% (±3.0). For progressive motility, the range was 2.21%–11.79% with a mean of 6.9% (±3.0).

# 4 | DISCUSSION

In this study, a strong correlation between Ongo and SpermVision sperm motility measurements was demonstrated. This was true for all three sperm concentrations studied. When comparing the different sperm concentrations analysed, the $50 \times 10^6$ sperm/ml concentration resulted in the highest r-values for progressive as well as total motility, followed by 25 and $100 \times 10^6$ sperm/ml. Ongo provides reliable results relative to SpermVision at all concentrations. This relationship was slightly stronger for progressive than for total motility.

Bland–Altman plots further suggest that Ongo and SpermVision provide similar measurement values. Agreement between the two methods was also strongest at the $50 \times 10^6$ sperm/ml concentration, confirming the correlation results. Limits of agreement were within a clinically acceptable range (Mortimer et al., 2015). The confidence intervals of the mean of the differences for progressive as well as total motility included the x-axis, indicating that there was no systemic bias between the two methods. Ongo and SpermVision can thus be used interchangeably at this concentration. Because $50 \times 10^6$ sperm/ml is in the recommended range for equine artificial insemination with cooled-stored semen (Brinsko, 2006; Jasko, Martin, & Squires, 1992; Varner, Blanchard, Love, Garcia, & Kenney, 1987b), the application of Ongo for semen analysis in an equine field practice can be recommended.

In veterinary field practice, quality assessment of a semen sample is usually based on estimation of total and progressive motility under a phase contrast microscope. In contrast to CASA systems used in the laboratory, the Ongo handheld device does not allow for a detailed analysis of individual sperm kinematic measurements like velocity or wobble characteristics, but categorizes spermatozoa according to WHO categories (WHO, 2010) into four categories. This may question the application of handheld devices like Ongo for scientific purposes. However, precision is also an important requirement for measuring devices used under field conditions. Precision, expressed as repeatability of measurements, was determined in an intra-assay variation trial. The relative SD, also known as coefficient of variation, was used to assess this quality control parameter. For this, the SD is divided by the mean and multiplied by 100, thus expressing the spread found in repeated measurements relative to the motility as a percentage. An average CoV across 10 samples, each measured 5 times, of 5.2% for total motility and 6.9% for progressive motility suggest a good level of precision for motility analysis results. In an evaluation of two CASA systems using boar semen at a concentration of $40 \times 10^6$ sperm/ml, one system (QualiSperm®, Biophos, Geneva, Switzerland) was found to have a similar CoV (6.0%), while another (SM-CMA®, Mika Medical GmbH, Rosenheim, Germany) resulted in 12.4% (Tejerina, Buranaamnuay, Saravia, Wallgren, & Rodriguez-Martinez, 2008).

Average motility determined by Ongo in $25 \times 10^6$ sperm/ml samples was lower than in samples with higher sperm concentrations and also lower than results obtained by SpermVision CASA. This may be caused by a longer interval between loading and completion of analysis compared to samples at higher concentrations. Because fewer cells are recorded and analysed per video, more videos have to be recorded with Ongo to reach 1,000 cells. Increased time until evaluation of an individual sample may lead to a decrease in motility (reviewed by Yeste et al., 2018). For future studies and practical application, it should thus be recommended to finish analysis within a given maximum time to ensure consistent motility of the sample during the analysis interval. In the present study, at the lowest concentration ($25 \times 10^6$ sperm/ml) recording videos of three fields yielded approximately 500 analysed cells in a total time of 2–3 min and no decrease in motility was observed within this time interval. The minimal requirement of 1,000 cells used in the present study was chosen for comparability with the CASA system but may not be necessary in a clinical setting under field conditions. Usually, evaluation of 500–1,000 cells is recommended, but there are studies where only 200–300 cells have been evaluated and resulted in satisfactory results (Yeste et al., 2018). Analysis of a total of 500 cells with Ongo may thus be considered acceptable under field conditions.

Standardized operating procedures as part of quality assurance are a necessity for the operation of CASA systems (Amann & Waberski, 2014; Broekhuijse, Šoštarić, Feitsma, & Gadella, 2011; Yeste et al., 2018) also when analysing stallion semen (Giaretta et al., 2017; Hoogewijs et al., 2012; Jasko et al., 1991; Love, 2011). Due to the automated operation of Ongo, standardization of measurements and associated training would be largely restricted to specimen preparation, thus limiting sources of variation associated with operating the measuring device. This will lead to greater consistency when more than one operator is using the equipment. Because training requirements are lower than for laboratory-based CASA systems, this also means that more individuals can easily carry out the task of motility analysis. The use of Ongo for semen analysis in a laboratory setting may be an option when financial limitations preclude the use of a CASA system.

In conclusion, Ongo sperm test can be a practicable, cost-effective and robust solution in cases where a full CASA system is not required, not practicable or too expensive. Such mobile semen analysis systems may bring quality assurance in animal breeding and livestock production further down the production chain.

## CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

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