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ORIGINAL ARTICLE

Evaluation of porcine semen quality by portable and desktop CASA systems – Short communication

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ABSTRACT

When using artificial insemination in porcine reproduction, one of the most important requirements is the suitable quality of semen regarding its total motility (TM) and progressive motility (PM). Computer-assisted sperm analysis (CASA) is an appropriate method to analyse the quality of semen. Recently a portable instrument has been developed to help specialists in their everyday field work. In our study, semen quality was measured simultaneously by the portable device (Ongo) and a laboratory CASA system (Microptic) to compare TM and PM values obtained by these appliances at a concentration of 50×10^6 spermatozoa/mL. Agreement between measurements was evaluated with a Bland-Altman plot. Strong correlation was found between the investigated instruments for all the three parameters, i.e. sperm concentration, TM and PM. However, a few measurements fell outside the defined range of acceptance.

KEYWORDS

semen quality, CASA, Ongo, Microptic

For a successful artificial insemination (AI) all quantity and quality parameters of semen and sperm cells have to be examined. It is generally accepted that a boar semen ejaculate with <60% total motility (TM) or >20% abnormalities may compromise fertility (Flowers, 2002).

Computer-assisted sperm analysis (CASA) is a widespread method which can objectively evaluate sperm motion characteristics, morphology and sperm concentration (SC). It provides an independent interpretation based on optical microscopy and 2D video micrography (Didion, 2008).

The accomplishment is highly dependent on the experience and routine of the examiner running the analysis (Buss et al., 2019).

In case of desktop CASA, semen evaluation is performed under laboratory conditions using a phase-contrast microscope connected to a desktop computer. A portable device can offer a rapid evaluation of semen under field conditions right after ejaculation or before insemination (Amann and Waberski, 2014).

Recently Buss et al. (2019) have examined semen from 10 stallions (diluted to three different concentrations, i.e. 25, 50 and 100 $\times$ 10^6 spermatozoa/mL) by a laboratory CASA system (SpermVision, Minitube, Tiefenbach, Germany) and a portable device (Ongo, Sperm Test®, Microfluidlabs, Budapest, Hungary) for TM and progressive motility (PM). When comparing the

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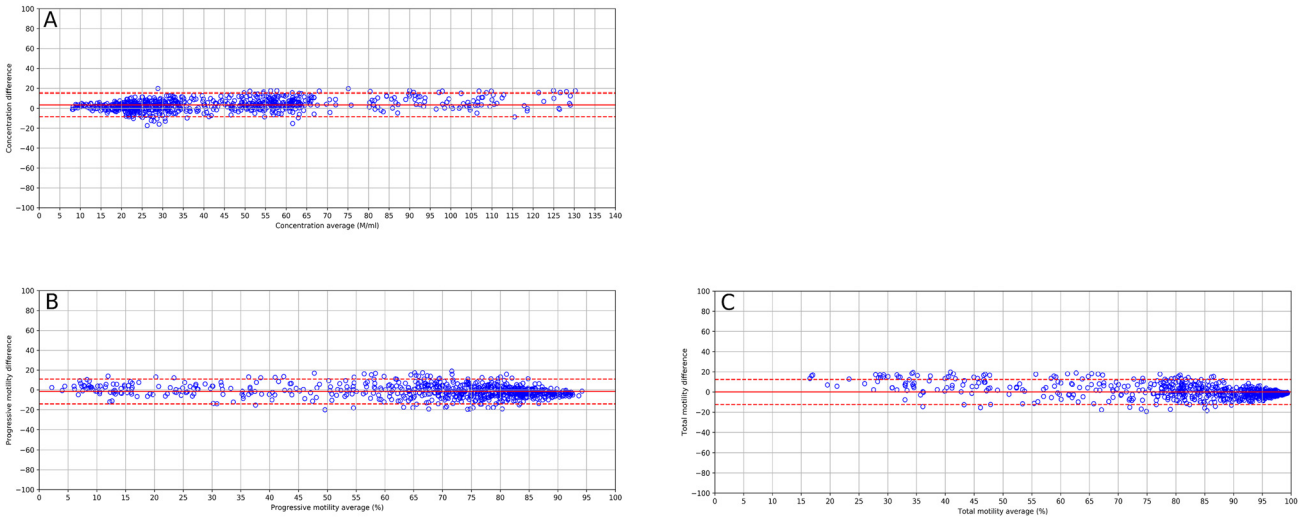

Fig. 1. Bland–Altman plots of measurements. The Y axis represents the difference between Ongo and Microptic CASA paired measurements. (A) Average sperm concentration (SC, M/mL) measured by Ongo Sperm Analyzer and Desktop (Microptic) CASA. The X axis shows the average SC of a given sample. The red line is the mean difference of concentrations at the Y value of 3.56 M/mL. Dashed, red lines are the upper and lower 95% limits of agreement at values 15.41 and −8.28, respectively. (B) Progressive motility (PM, %) measured by Ongo Sperm Analyzer and Desktop (Microptic) CASA. The X axis shows the average of PM (%) of a given sample. The red line is the mean difference of PM at a value of −1.49%. Dashed, red lines are the upper and lower 95% limits of agreement at values 11.00 and −13.98, respectively. (C) Total motility (TM, %) measured by Ongo Sperm Analyzer and Desktop (Microptic) CASA. The X axis shows the average TM (%) of a given sample. The red line is the mean difference of TM at value 0.00%. Dashed, red lines are the upper and lower 95% limits of agreement at values 12.42 and −12.43, respectively

analysed SCs, the concentration of 50×10^6 spermatozoa/mL resulted in the highest r values for PM and TM. Agreement between the results was evaluated by a Bland–Altman plot. The results obtained by the portable CASA strongly correlated with those obtained by desktop CASA.

In this study we assessed boar semen motility using the previously mentioned portable device and a desktop CASA in order to compare the results of these two instruments and to investigate the reliability of measurements obtained by the portable device.

A total of eight boars (two Duroc × Pietrain, two Danbred × Duroc, two Hungarian Landrace and two Hungarian Large White boars) were included in the study and 1,164 semen samples were collected from these boars using a gloved-hand technique (Althouse et al., 2006). The freshly ejaculated boar semen was diluted with Beltsville Thawing Solution (BTS) (Pursel and Johnson, 1975) and examined right after collection. The delivery temperature was 17 °C. All ejaculates were analysed for concentration, TM and PM by a desktop CASA system (Sperm Class Analyzer – SCA, Microptic S. L., Barcelona, Spain) and a portable device (Ongo Sperm Test®, Microfluidlabs, Budapest, Hungary).

Every sample was analysed for SC, PM and TM by Ongo and Microptic CASA to compare the two systems. The measuring process was always the same. The samples were diluted with BTS to the final concentrations of 50×10^6 spermatozoa/mL. Ten µL of diluted semen was pipetted into the chambers of the slide. The classification of spermatozoa was performed according to Buss et al. (2019). Statistical analysis was performed by the use of a Bland–Altman plot (Bland and Altman, 1986), where differences between measured values (paired values) are plotted against the mean of the paired values. Plotting and statistical calculations were performed with the Python 3.6.2 software (Python Software Foundation, Wilmington, Delaware, United States).

After the examination of the samples a total of 1,164 values were obtained on both Ongo and Microptic CASA. SC agreement (Fig. 1A) was found at Ongo vs. Microptic CASA, the bias was 3.56 M/mL, with a 95% limit of agreement (upper limit = 15.41 M/mL, lower limit = −8.28 M/mL). The bias of PM was −1.49%, with a 95% limit of agreement (upper limit = 11.00; lower limit = −13.98) (Fig. 1B). The mean difference of TM (Fig. 1C) was 0.00%, with a 95% limit of agreement (upper limit = 12.42; lower limit = −12.43).

In semen analysis the values measured by a clinical andrology laboratory must be within ±10% of the reference values, whereas a ±20% deviation might be acceptable for a general diagnostic laboratory (Mortimer et al., 2015). In our study the ratios of measurement falling outside the defined range of acceptance were low. At a general diagnostic laboratory level 2.15, 2.41 and 1.72% of the Ongo values were outside the defined range of acceptance in case of SC, PM

Table 1. Percentage of Ongo measurements falling outside the defined range of acceptance

Defined range of acceptance (%)	Sperm concentration	Progressive motility	Total motility
20	2.15	2.41	1.72
30	0.17	1.03	0.34

and TM, respectively. The same values were lower at a relaxed range (Table 1), which is more suitable for a portable device.

A strong agreement was demonstrated between results obtained by Ongo and Microptic CASA devices concerning SC, PM and TM.

Average differences for SC, PM and TM represented on Bland–Altman plots indicate a slight bias between the two instruments regarding SC and PM. Low ratios of measurements fell outside the defined range of acceptance at the general diagnostic laboratory level. These ratios dropped at a more relaxed level (Table 1).

According to the outcome of this study SC, PM and TM results of spermatozoa obtained by the Ongo portable device are similar to those provided by the Microptic desktop CASA system. The Ongo instrument is a practical and cost-effective opportunity in cases where a complete CASA system is not available or not affordable. Ongo can be recommended as a fast appliance for semen analysis in the field practice of animal breeding and veterinary medicine, but the results must be evaluated on the field level.

Although correlations among the three parameters were found (Fig. 1), still we recorded a few measurements which were outside the defined range of acceptance (Table 1). We assume that more accurate results could be achieved if the boar-semen-specific configuration of the Ongo instrument were further improved.

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