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Comparison of FACSCount AF system, Improved Neubauer hemocytometer, Corning 254 photometer, SpermVision, UltiMate and NucleoCounter SP-100 for determination of sperm concentration of boar semen

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Abstract

Current research aims at reducing the number of sperm per insemination dose thereby making measurement of sperm concentration in raw semen and the production of uniform insemination doses much more crucial.

The present study evaluated the determination of sperm concentration using FACSCount AF System (FACS), Improved Neubauer hemocytometer (HEMO), Corning 254 photometer (Photo C254), SpermVision CASA System (SpermVision), UltiMate CASA System (UltiMate) and NucleoCounter SP-100 (SP-100). The instruments were evaluated with respect to repeatability and to establishing the regression curve towards both HEMO and FACS.

Repeatability for the instruments was 2.7, 7.1, 10.4, 8.1, 5.4 and 3.1% for FACS, HEMO, Photo C254, SpermVision, UltiMate and SP-100, respectively. Correlation between instruments was highest between FACS and SP-100. This was made possible due to the high repeatability for both instruments. The agreement between the instruments and HEMO as the gold standard was lower than expected as the largest difference in estimation of concentration was −25 to +50%. The largest percentage difference was observed for measurements of dilute semen. It was clear that percentage difference between instruments depended on sperm concentration. In comparison to the gold standard, agreement was highest between SpermVision and HEMO for dilute semen, but for concentrated semen, agreement was highest between SP-100 and HEMO. However, the agreement between HEMO and all other instruments was not as good as expected. The reason may lie within the presence of agglutinated sperm, preventing proper HEMO counts.

Keywords: Sperm concentration; Semen; Boar; Reproduction; CASA; Counting chamber

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1. Introduction

The accurate and precise determination of sperm concentration is important for AI-stations in order to produce uniform insemination doses. The uniform insemination dose becomes even more important as present research aims at reducing number of sperm per insemination dose [1]. All steps in the production line contribute to the variation in the number of sperm in insemination doses and therefore attention has been drawn to the sperm concentration measurement of raw semen.

The World Health Organization specifies that the most accurate method for determination of sperm concentration is by the use of a hemocytometer, which is considered to be the gold standard [2]. However, the hemocytometer is not applicable to routine measurement of sperm concentration as it is laborious and slow. Past the years, work has been aimed at establishing an automated faster method for determination of sperm concentration. Instruments have been compared and validated against hemocytometers and it has often been shown that there was a difference between the hemocytometer and the instrument.

There are examples of validation using counts of either beads [3–5] or sperm [6,7]. Additionally, validation has been performed using photographic documentation of beads [8].

Most of the studies show some differences between the gold standard and the instruments.

Differences between instruments and gold standard are to be expected. Evaluating accuracy for an instrument, however, is merely the comparison of the instrument with the gold standard. Such comparisons give information on repeatability, correlation between instrument and gold standard and lastly the regression curve between the instruments and the gold standard. The regression curve serves as the mathematical correction factor needed to transform the result into the correct sperm concentration.

The present study was performed to evaluate the determination of sperm concentration using FACSCount AF System (FACS), which is a flow cytometer, Improved Neubauer hemocytometer (HEMO), Corning 254 photometer (Photo C254), SpermVision CASA System (SpermVision), UltiMate CASA System (UltiMate) and NucleoCounter SP-100 (SP-100), which is a novel counting chamber based instrument. Instruments were evaluated with respect to repeatability and to establishing the regression curve towards both HEMO and FACS. Comparison with HEMO was chosen, since HEMO remains the gold standard; FACS was chosen due to the very high repeatability, which was shown in previous work [6].

2. Materials and methods

2.1. Experimental design

Raw semen from 161 different DanBred boars on DanBred AI-stations was collected as whole ejaculate collection. A sample of approximately 10 mL of raw semen was transferred to a 13 mL Nunc tube (NUNC A/S, Roskilde, Denmark). This sample was used for the concentration measurements for all instruments. Room temperature was maintained at 20 °C during the trial.

For each instrument the sperm concentration was measured in duplicates by the same technician. All steps in the concentration measurement were performed for each measurement.

2.1.1. Sperm concentration measurement using the FACSCount AF System

The sperm concentration measurement using the FACSCount AF System (BD Biosciences, Franklin Lakes, NJ) was based on an internal standard of fluorescent beads. The diluted semen sample was added to a counting tube. Each counting tube contained standardised fluorescent beads of specific concentration calibrated by Becton Dickinson. When the sample was analysed in the flow cytometer, both beads and sperm were counted. The number of beads in the analysis was used to calculate the concentration of sperm. Settings for the instrument was to analyse 15,000 events or stop after 30 s.

Preparation of sperm counting reagent and analysis on the FACS was performed as described by Christensen et al. [9].

2.1.1.1. Dilution of semen. A volume of 5.0 mL EDTA boar semen extender (LEO Pharma, Ballerup, Denmark) was added to a 10 mL PP-tube (Hounisen laboratorieudstyr A/S, Risskov, Denmark) using a DispensetteIII (Brand, Wertheim, Germany). After careful mixing of the raw semen, 50 vL was added to the PP-tube using a FACSCount pipette (BD Biosciences). Pipetting was performed using reverse pipetting. After mixing of the diluted semen, 50 vL was transferred to the counting tubes using the FACSCount pipette. All pipetting was performed with wiping the pipette tip carefully using a Kim-Wipe (Kimberly-Clark Nordic, Bagsvaerd, Denmark) before adding the pipetted volume.

The counting tube was incubated for 5 min at room temperature before analysis in the flow cytometer.