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Technical note

Is photometry an accurate and reliable method to assess boar semen concentration?

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Abstract

Sperm concentration assessment is a key point to insure appropriate sperm number per dose in species subjected to artificial insemination (AI). The aim of the present study was to evaluate the accuracy and reliability of two commercially available photometers, AccuCell™ and AccuRead™ pre-calibrated for boar semen in comparison to UltiMate™ boar version 12.3D, NucleoCounter SP100 and Thoma hemacytometer. For each type of instrument, concentration was measured on 34 boar semen samples in quadruplicate and agreement between measurements and instruments were evaluated. Accuracy for both photometers was illustrated by mean of percentage differences to the general mean. It was −0.6% and 0.5% for Accucell™ and Accuread™ respectively, no significant differences were found between instrument and mean of measurement among all equipment. Repeatability for both photometers was 1.8% and 3.2% for AccuCell™ and AccuRead™ respectively. Low differences were observed between instruments (confidence interval 3%) except when hemacytometer was used as a reference. Even though hemacytometer is considered worldwide as the gold standard, it is the more variable instrument (confidence interval 7.1%).

The conclusion is that routine photometry measures of raw semen concentration are reliable, accurate and precise using AccuRead™ or AccuCell™. There are multiple steps in semen processing that can induce sperm loss and therefore increase differences between theoretical and real sperm numbers in doses. Potential biases that depend on the workflow but not on the initial photometric measure of semen concentration are discussed.

Keywords: Reproduction; Sperm concentration; Photometer; Boar; Semen

1. Introduction

Animal species of agricultural interest are mainly produced by artificial insemination. The efficiency of this method (fertility rate and prolificacy) is directly dependent on the quality of semen doses and on the number of spermatozoa used for insemination [1]. Evaluation of concentration is crucial to adapt dilution rate and to optimize sperm concentration which will directly impact fertility performance. Powerful instruments are available for AI production centers to assess both quality and concentration of semen. The Computer Assisted Semen Analyzer (CASA) evaluates the concentration of semen sample and the kinematics of sperm cells. Cytometers and flow-cytometers with fluorescent dyes evaluate concentration and/or the sperm parameters related to sperm physiology. The minimum requirement is visual evaluation by bright field microscopy and concentration assessment with hemacytometer or photometers.

Accurate concentration measurement is the first and a crucial step of the semen preparation process for production of semen doses with desired number of

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spermatozoa per dose. However, the following steps in semen processing may also impact the target number of spermatozoa per dose if they are not properly performed.

The accuracy, reliability and repeatability of different instruments that evaluate sperm concentration of raw semen have already been compared in several previous studies [2–5]. Repeatability of assessments is reported as average coefficient of variation (CV) with values ranging from 4.1% [3] to 10.4% [2] depending on instruments and procedures.

The aim of this study was to evaluate the accuracy and the reliability of two photometers, the Accucell™ and the AccuRead™ (both distributed by IMV Technologies, L'Aigle, France), that have been developed for boar semen concentration assessment but use different optical systems. UltiMate™ boar version 12.3D CASA system (Hamilton Thorne Biosciences, USA), NucleoCounter SP100 (ChemoMetec, A/S, Allerød, Denmark) and Thoma hemacytometers double, depth 0.1mm, grid 0.05mm (ROGO-SAMPAIC, Wissous, France) were chosen as instruments for comparison. For both photometer models, variations between at least 10 devices were quantified in order to estimate whether individual calibration procedures were necessary. The study also aims to show that calibration can be standardized.

2. Materials and methods

2.1. Optical system and measurement preset protocol

2.1.1. AccuCell™

The light source of the AccuCell™ is a pre-adjusted halogen lamp. The light is dispersed by a prism into different colors in a range of the optical spectrum. This photometer automatically calibrates using an internal didymium filter. The wavelength sets automatically to 530 nm. The spot size of the optical beam is about 1.5 × 2.5 mm with slight variations between instruments.

AccuCell™ measures during 6 seconds at two readings per second. The reported result is the average of 12 readings.

2.1.2. AccuRead™

The light source of the AccuRead™ is a 595 nm LED with an optical beam diameter defined by an optical fiber. The diameter of the spot is 3 mm, and is constant among AccuReads™.

AccuRead™ performs one single measurement over a 2 second period.

2.2. Calibration of the photometers

The two types of photometers were first calibrated with 60 semen samples collected from two boar studs. Semen concentrations ranged from 70 to 1444 million sperm cells per mL. Reference assessments for sperm concentration were performed with the UltiMate™ (Hamilton Thorne Biosciences) and the NucleoCounter SP100 (ChemoMetec, A/S, Allerød, Denmark) with both instruments previously validated with Thoma hemacytometer as a gold standard with minimum 400 sperm cells counted per sample [6–8]. For the UltiMate™, the algorithm used to calculate concentrations includes appropriate compensation factors to adjust for the Segre-Silberberg effect [9–11]. The regression determined between absorbance and concentration was a curvilinear of third degree equation ($y = ax^3 + bx^2 + cx + d$) with a correlation coefficient of $r^2 = 0.97$.

2.3. Experimental design

One single ejaculate was collected from thirty-four Pietrain boars at a commercial insemination center (Amélis, Barenton, France). Immediately after collection, ejaculates were homogenized (gentle shaking for 30 seconds) and then a 100 μL aliquot was diluted in 2.4 mL of TriXcell™ (IMV Technologies, l'Aigle France) in a disposable plastic cuvette for photometers (IMV Technologies) and closed with Parafilm™ (Pechiney, Chicago, USA). Every diluted sample was then assessed with each instrument as described below.

2.3.1. Sperm concentration assessment with AccuCell™ and AccuRead™

Each of 34 Pietrain boars semen samples were first diluted 1:24 (v:v) using TriXcell extender, cuvettes were then read four times for absorbance and concentration with each of both photometers tested (AccuCell™, AccuRead™). Samples were turned upside down twice between measurements, which occur within 5 seconds after the placement of the cuvette inside the photometer. In a second part of the study, the reproducibility of absorbance measurement was tested using 12 AccuCells™, (each of them being filled successively with 11 samples of diluted semen) and 10 AccuReads™ (each of them being filled successively with 39 samples of diluted semen).

2.3.2. Sperm concentration assessment with the UltiMate™ CASA system

Semen samples were analyzed with the UltiMate™ four hours after the first semen collection. Prior to analysis, the cuvette samples were shaken upside down twice and 200 μL were pipetted into polypropylene 1.5 mL microtubes (ref B29012 Fischer Bioblock Scien-