Influence of Technical Settings on CASA Motility Parameters of Frozen Thawed Stallion Semen

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The repeatability of the analysis of frozen thawed equine semen using a computer-assisted sperm analysis system is high. However, the settings used to analyze the semen samples influence the results to a large extent. Therefore, settings used in different studies should be described clearly, facilitating comparison of results. Authors’ address: Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133 B-9820, Belgium; e-mail: maarten.hoogewijs@UGent.be (Hoogewijs). © 2009 AAEP.

1. Introduction

The widespread use of frozen thawed semen in the equine breeding industry creates opportunities for breeders and stallion owners. Semen can be shipped all over the world so that any stallion may become available for any given mare. Inseminations with frozen thawed semen, however, are less successful compared with inseminations with cooled and, especially, fresh semen. To optimize pregnancy outcome, frozen thawed semen should fulfill some criteria such as a minimal total number of spermatozoa per dose and a minimal progressive motility, e.g., the insemination dose for conventional artificial insemination (AI) using frozen thawed semen should at least contain 240 × 10⁶ progressively motile spermatozoa (PMS).¹ The progressive motility (PM) should at least be 30%,¹ whereas the World Breeding Federation for Sport Horses advocates a PM of 35%.²

Computer-assisted sperm analysis (CASA) is accepted as an accurate tool for motility analysis. A number of motility settings for CASA have been described. In this paper, we studied the repeatability of CASA. Furthermore, the influence of CASA motility settings on several sperm parameters when analyzing frozen thawed semen with two different settings was studied.

2. Materials and Methods

The semen used in this experiment was frozen in 0.5-ml straws at two European-approved AI centers in Belgium. Straws (n = 63) of different Warmblood and Arabian stallions were thawed in a water bath of 37–38°C for 30 s, dried, and emptied in a small cup. An aliquot of 200 µl was diluted to a final concentration of 35–40 × 10⁶/ml in a good quality semen extender, based on modified Hanks’ salts supplemented with native phosphocaseinate³
free of debris when visualized microscopically. The diluted samples were incubated at 37–38°C for 10 min before analysis. Each sample was analyzed with a CASA system \(^b\) using two different settings (CASA1 and CASA2). Settings for CASA analysis were obtained from established semen processing laboratories and are routinely applied to fresh, cooled, and frozen-thawed semen samples.

The major settings used for CASA1 were as follows: straightness (STR) threshold for progressive motility, 75%; average path velocity (VAP) threshold for progressive motility, 50 \(\mu\text{m/s}\); VAP threshold for static cells, 20 \(\mu\text{m/s}\).\(^3\) These settings were first obtained after a personal communication, and settings for cell detection were adapted for optimal sperm recognition. The major settings used for CASA2 were as follows: STR threshold for progressive motility, 50%; VAP threshold for progressive motility, 30 \(\mu\text{m/s}\); VAP threshold for static cells, 15 \(\mu\text{m/s}\); settings for cell detection are listed as well.\(^4\)

Six microliters of diluted semen was loaded into a 20-\(\mu\text{m}\) counting chamber, \(^c\) placed on a stage warmer \(^d\) during the analysis. Five different fields were analyzed four times to obtain a total of 20 fields to obtain a minimum of 1500 cells analyzed. For both CASA settings, this was done twice per sample.

The parameters analyzed were percentage total motility (TM), PM, and PMS (normally distributed). To assess repeatability, each sample was analyzed twice within 1 min (paired-samples t-test). To assess agreement of settings, each sample was analyzed using settings CASA1 and CASA2 (paired-samples t-test). Statistical analysis was done using SPSS17.

3. Results

Analyzing the semen samples using CASA1 and CASA2 was highly repeatable (\(p \geq 0.082\); \(r \geq 0.97\)). For CASA1, TM, PM, and PMS were \(38.0 \pm 12.2\%\), \(21.3 \pm 10.2\%\), and \(36.4 \pm 19.2 \times 10^6\) at the first analysis and \(37.3 \pm 12.4\%\), \(21.6 \pm 1.0.3\%\), and \(35.9 \pm 17.6 \times 10^6\) at the second analysis. For CASA2, these values were \(30.6 \pm 12.6\%\), \(28.3 \pm 12.2\%\), and \(41.9 \pm 21.2 \times 10^6\) at the first analysis and \(30.4 \pm 12.7\%\), \(28.1 \pm 12.2\%\), and \(42.1 \pm 21.4 \times 10^6\) at the second analysis.

The CASA settings influenced the results substantially when analyzing the three sperm quality parameters (\(p \geq 0.001\); \(r \geq 0.97\)). Average TM, PM, and PMS for CASA1 and CASA2 were \(37.7 \pm 12.3\%\), \(21.4 \pm 10.2\%\), and \(36.1 \pm 18.3 \times 10^6\) and \(30.5 \pm 12.6\%\), \(28.2 \pm 12.2\%\), and \(42.0 \pm 21.2 \times 10^6\), respectively.

4. Discussion

The importance of indicating which CASA settings are used when assessing the quality of frozen thawed semen is clearly shown. Depending on the settings used, an ejaculate might be discarded or accepted for use in an AI program. As shown in this study, when quality of semen samples is reported, it is important to mention how this semen was analyzed and describe precisely which settings were used.

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References and Footnotes


\(^a\)INRA96, IMV Technologies, 61302 L’Aigle, Cedex, France.
\(^b\)CEKOS, Hamilton-Thorne Inc., Beverly, MA 01915.
\(^c\)Leja, 20-\(\mu\text{m}\) counting chamber, Leja, 2153 GN Nieuw-Vennd, The Netherlands.
\(^d\)Minitherm stage warmer, Hamilton-Thorne Inc., Beverly, MA 01915.