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Abstract

Validation of an objective approach for simultaneous assessment of viability and motility of fresh and cooled equine spermatozoa☆

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1. Introduction

Routine semen analysis is a valuable instrument in the qualitative determination of an ejaculate's fertilizing potential. There are many techniques for assessing the viability and motility of spermatozoa separately but not, to date, in combination. The main objective of this study was to refine and validate a rapid procedure for concurrent assessment of the viability and motility of cooled equine spermatozoa.

2. Materials and methods

Two ejaculates from each of seven stallions were collected with a Missouri model artificial vagina, assessed and extended to a concentration of 50 million/ml in a non-fat dried skim milk (NFDSM) extender (Kenney's) with Ticarcillin. Samples were evaluated at 0, 24 and 48 h after cooling at 5 °C in a Hamilton–Thorn Equitainer©. Semen was warmed for 10 min at 37 °C and evaluated using a computerized semen analysis system (IVOS, v 12.2, Hamilton Thorn Research, Beverly, MA). Total and progressive motility and viability of each sample were determined using the "VIADENT" option. The Viadent stain (Hoechst 33258) is a vital stain that marks only the cells with non-intact membranes. The VIADENT option uses visible (blue light emitting diode)

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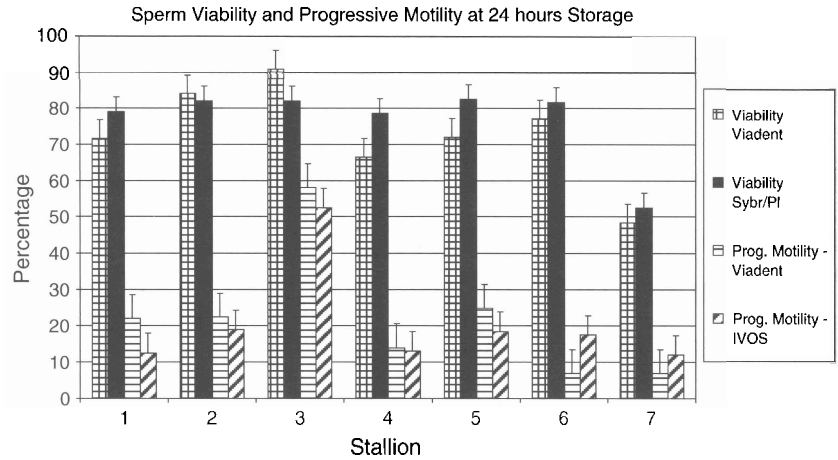

Fig. 1. Sperm viability and progressive motility assessed with the IVOS system from seven different stallions.

light to determine cell count and motility prior to using fluorescent light to determine the number of non-viable cells. For viability comparison, SYBR-14/propidium iodide analysis was performed in tandem with all VIADENT tested samples. Total and progressive motility were assessed in both the "Viadent" stained samples and the spermatozoa that were extended in the NFDSM extender. A minimum of 200 sperms were evaluated for each measurement. Viability results between these two techniques were compared using ANOVA.

3. Results

The data show differences in viability and motility between stallions. Total viability of the ejaculates ranged from 31% to 95%. A decrease in viability, total and progressive motility was observed over time. Viability measured by the IVOS system with the "Viadent" stain was found to be similar to the percentage of viable spermatozoa measured by fluorescent microscopy and the PI/SYBR-14 dye ($P < 0.05$) (Fig. 1).

4. Discussion

Garner et al. (1994) established the effectiveness of the membrane permeant DNA stain, SYBR-14, in assessing the viability of fresh and frozen bull spermatozoa. Subsequently, this technique was validated by Merkies et al. (2000) for horses. Our results suggest that the "Viadent" option of the IVOS system is capable of a rapid, accurate and objective evaluation of both viability and motility parameters using large numbers of spermatozoa. Application of this technique in the industry may prove useful in the clinical assessment of fertilizing potential of equine spermatozoa.

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