

Sperm motility and progressive motility were evaluated with B-SpermTM computerized video software (Medical Electronic Systems, Ltd. Caesarea Industrial Park, Israel). Bull semen was visualized “live” on a PC monitor using the video image mode; non-motile and non-progressively motile spermatozoa were counted first, then the “live” image was “frozen” and all sperm were counted. Percentages of motility and progressive motility were calculated. Each semen sample was counted twice (minimum of 100 spermatozoa per replicate). For precision, coefficients of variation (CV) were calculated based on duplicate samples. For analytical accuracy, correlations were determined between the automated versus the manual method. Data are summarized below (Table 1).

Coefficients of variation $\leq 10\%$ demonstrated high repeatability of the SQA-Vb. Furthermore, SQA-Vb and manual data were highly correlated. Therefore, we concluded that the SQA-Vb was a viable alternative to manual analysis of bull semen.

Keywords: SQA-Vb; Semen analysis; Motility; Sperm; Cattle

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Assessing cryocapacitation in frozen-thawed bovine sperm from a commercial bull stud

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Precocious sperm capacitation or ‘cryocapacitation’ may contribute to reduced fertility for cryopreserved semen, but there is no quantifiable, biochemical assay for this functional change. We hypothesized that if frozen-thawed bovine sperm undergo cryocapacitation, it would be detected by protein tyrosine phosphorylation, a quantifiable biochemical marker of fresh bovine sperm capacitation, and an increase in the rate of lysophosphatidylcholine-induced acrosome reaction (LC AR), a standard sperm capacitation assay. For this study, commercially processed cryopreserved semen (milk-glycerol extender) from single ejaculates of 19 Holstein bulls was evaluated twice for each bull. Samples were evaluated post-thaw (0 h) or after a 4-h incubation in TALP medium with: no additives (NA); or 10 $\mu\text{g/mL}$ heparin (H); or 10 $\mu\text{g/mL}$ heparin and 1 mM dibutyryl-cAMP plus 100 μM IBMX (HcA; known to

maximize bovine sperm capacitation). Sperm were assessed for viability using CFDA/Propidium Iodide staining, for spontaneous (sAR) and LC AR (Coomassie G-250 stain), and for protein tyrosine phosphorylation, as assessed by both antiphosphotyrosine slot blot analysis and standard one-dimensional SDS-PAGE immunoblot analysis. Protein tyrosine phosphorylation of slots and one band (p48) were quantified by image analysis (Gel-Pro 4.5, Media Cybernetics, Silver Spring, MD, USA). Data are presented as means $\pm$ S.D. S.D. and were evaluated by three-way ANOVA, followed by Tukey’s test for multiple comparisons, with significance set at $P < 0.05$ (Sigma-Stat 3.0; SPSS Inc., Chicago, IL, USA). Except for two bulls with low viability (A, B), there were no significant inter-bull differences in sperm viability, but viability declined in significant increments for NA, H, and HcA (0 h: $60.7 \pm 8.9\%$; NA: $53.0 \pm 8.2\%$; H: 47.8 ± 10.3 ; and HcA: $33.3 \pm 8.9\%$). The rates of sAR at 0 h were lower than the 4 h incubated samples (0 h: $15.7 \pm 6.6\%$; NA: $40.6 \pm 21.3\%$; H: 42.7 ± 19.8 ; HcA: $39.6 \pm 16.9\%$) and only two bulls (B, C) had significantly higher sAR rates. Rates of LC AR at 0 h were highly variable, did not differ significantly between bulls or between experiments for the same bull, and were consistent with some cryocapacitation ($14.8 \pm 17.1\%$). LC AR at 4 h was significantly reduced compared to 0 h, highly variable, and not consistently repeatable (NA: $1.7 \pm 17.7\%$; H: -1.3 ± 13.8 ; HcA: $1.4 \pm 10.2\%$). Slot blot analysis was consistently repeatable for 0 h samples, but not for incubated samples, and detected significantly higher protein tyrosine phosphorylation for one bull (A). Tyrosine phosphorylation of p48 relative to positive control (HcA), differed significantly among treatments (0 h: $17.8 \pm 7.8\%$; NA: $27.5 \pm 10.7\%$; H: $33.3 \pm 13.5\%$), was consistently repeatable, and detected two bulls (D, E) with reduced, and one with increased (F) p48 tyrosine phosphorylation. The relative lack of inter-bull differences detected with these assays was not unexpected, as all bulls were proven sires. Tyrosine phosphorylation of p48 provided data similar to LC AR at 0 h, but not following incubation, perhaps due to the high rate of sAR. Protein tyrosine phosphorylation is therefore a good candidate biochemical marker for bovine sperm cryocapacitation, but should be characterized in bulls with more variable responses to freezing.

Keywords: Semen analysis; Sperm; Cryocapacitation; Tyrosine phosphorylation; Bovine

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