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## JOURNAL ARTICLE

# Dual DNA staining assessment of bovine sperm viability using SYBR-14 and propidium iodide

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A new membrane-permeant DNA stain, SYBR-14, was used in combination with propidium iodide (PI) to estimate the proportion of living sperm in bovine semen. The SYBR-14 stained living sperm while PI only stained degenerate cells that had lost their membrane integrity. Staining with SYBR-14 resulted in the nuclei of living sperm fluorescing bright green. Aliquots containing nearly all living bovine sperm were prepared using glass wool/Sephadex filtration to remove dead and damaged cells. A portion of this filtered sample was killed by unprotected freeze-thawing and used to provide mixed aliquots containing known ratios of living and dead sperm. Flow cytometry was used to assess the green and red fluorescence of these mixtures. The percentages of living sperm, as determined by the log of green fluorescence, were 85.1, 68.8, 39.8, 20.7, and 1.4 for ratios of 100:0, 75:25, 50:50, 25:75, and 0:100 of the filtered, killed mixtures. Also, bovine semen was diluted 1:60 in HEPES-0.1% bovine serum albumin and incubated for 0, 3, 6, and 24 hours at 36 degrees C to assess changes in cell viability. As cell death occurred during this incubation period, a relatively rapid transition of staining from green to red occurred as sperm died. Three replicates of cryopreserved sperm from six bulls were also examined using SYBR-14 and PI to assess the proportion of living and dead cells. Flow cytometric analyses of these samples, which had been processed and stored in homogenized milk, indicated that this stain combination was useful in assessing the quality of cryopreserved sperm. The combination of SYBR-14 and PI was determined to be an effective tool for assessing the viability of fresh or cryopreserved sperm.

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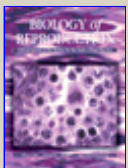
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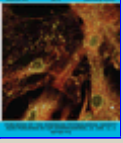
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