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A Flow Cytometric Method for Rapid Determination of Sperm Concentration and Viability in Mammalian and Avian Semen

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A new flow cytometric method has been developed to rapidly determine sperm concentration and viability in semen from bulls and boars. Sperm viability was determined on the basis of staining with SYBR-14 and propidium iodide (PI), and this allowed detection of live (membrane-intact) sperm, dying (moribund) sperm, as well as dead cells. Fluorescent microspheres (beads) were used to determine sperm concentration. The use of SYBR-14 at 50 nM and PI at 12 µM in combination with the FACSCount diluent in the counting tubes resulted in a uniform staining after 2.5–5 minutes at room temperature. Reagent staining was reproducible enough to allow subsequent semiautomated analysis of data using Attractors software. In experiment 1, this method was used to analyze semen from boars, rams, rats, rabbits, humans, and turkeys. In experiment 2, Attractors analysis was performed by the FACSCount AF flow cytometer, and sperm concentration determination with this system was compared with results obtained by a spectrophotometer and an electronic cell counter, which is routinely used by bull artificial insemination centers. When compared to microscopic counting in a hemocytometer, the FACSCount AF flow cytometer was two and four times more accurate than the spectrophotometer and the electronic cell counter, respectively. In addition, the FACSCount AF flow cytometer determined both sperm concentration (coefficient of variation 3.3%) and sperm viability (coefficient of variation 0.7%) with high precision.

Key words: Cytometry, spermatozoa, count, vitality, precision, accuracy

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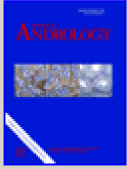

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