DETERMINING BULL SPERM CONCENTRATION: A NOVEL DESIGN FOR CASA

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Sperm cell concentration is a crucial quantitative measurement routinely taken on samples of bull semen. An accurate measurement allows semen to be efficiently utilized for optimum artificial insemination (AI) without wasting sperm of commercial value (Foote, 1972). Several instruments and methods can be used to determine sperm cell concentration, such as hemacytometer counts, spectrophotometrical densities, electronic cell counters, computer assisted semen analyzers (CASA), and flow cytometry. CASA use has been limited because of lack of characterization of individual systems and the absence of standardizations between laboratories. This study was conducted to: (1) test the use of a hemacytometer as a counting chamber for use with the Hamilton Thorn CASA system for AI and research settings, and (2) compare three different methods for the determination of sperm cell concentration in bull semen. Once weekly for three weeks, two ejaculates (20 min apart) were collected from each of three Holstein bulls using an artificial vagina. Triplicate samples of 2 ml per ejaculate were used for each analysis. Sperm cell concentrations were determined by using a hemacytometer and the following formula: concentration (million/ml) = cells counted $\times$ dilution ratio $\times$ hemacytometer factor (50) $\times$ 1000. The hemacytometer factor (HF) is based upon its physical characteristics: the grid area of 1 mm^2 and a depth of 0.1 mm to yield a chamber volume of 0.1 mm^3; the 80 square area where sperm cells were counted is then divided by the total area (400 squares). This result is multiplied by chamber volume and the inverse of this product is the HF. To determine sperm cell concentrations with CASA, the hemacytometer was used as a counting chamber, and the area detected by the computer system was measured. The detectable grid area, 0.17395 mm^2, was measured with a stage micrometer. This area was transformed to squares, and the number of squares in the measured area was divided by the total number of squares in the grid. This product was then multiplied by the chamber depth, and the inverse of the final product was the modified HF to be used with the CASA system. The nucleic acid stain SYTO® BC and a known concentration of fluorescent beads were used in calculating sperm concentration with flow cytometry. Data were analyzed by least squares methods. The flow cytometer method differed ($P < 0.0027$) from the other two methods at week one. There was no difference between the hemacytometer and the CASA system when the hemocytometer was used as a counting chamber ($P > 0.05$), and the two methods were highly correlated ($r = 0.98$). From this study, it was concluded that the hemacytometer can be used in conjunction with CASA systems as a substitute for the commercially available disposable counting chambers and time used in counting will be decreased. Because some variation existed among these methods, there is room for further optimizations between the methods.

Keywords: Sperm; Concentration; Hemacytometer; Flow cytometry; CASA