Computer-assisted semen analysis (CASA) to determine sperm concentration: a comparison of slide preparation methods using the Hamilton-Thorne and Minitube systems

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Abstract

This study was conducted to test the hypothesis that different slide preparations will provide accurate sperm concentration values when evaluated using CASA systems. The sperm concentration of samples prepared using six different slide preparation methods were evaluated using two CASA machines (Hamilton-Thorne TOX IVOS II; Hamilton Thorne, Inc., Beverly, MA) and Minitube SpermVision Therio Version (MOFA, Verona, WI). The slide preparation methods included a DRM-600 CELL-VU® Sperm Counting Chamber (Millenium Sciences Inc., New York, NY), using a 22 x 22 mm #2 cover slip (Method 1); - a plain glass microscope slide using a 22 x 22 mm #2 cover slip with 7 uL of semen (Method 2); - a plain glass slide using an 18 x 18 mm #1 cover slip with 7 uL of semen (Method 3); - a plain glass slide using a #2 cover slip with a “hanging drop” from a Pasteur pipette (Fisher Scientific, Waltham, MA; Method 4); - a Leja counting chamber (IMV International Corp., Maple Grove, MN) (Method 5); and a Minitube standard counting chamber (Method 6). Each method was run with samples of known sperm concentrations (230 million sperm/mL, 115 million sperm/mL and 57 million sperm/mL). These six methods were evaluated for precision when compared to one another and accuracy when compared to the known sample. The concentration of the known sample was determined by hemacytometer count (Method 7), and verified by a nucleo-counter and densimeter (control). For sperm at 230 million cells/mL, the average sperm concentrations and standard deviations determined for each method were 111 ± 21, 141 ± 53, 161 ± 46, 316 ± 101, 276 ± 20, 289 ± 31, and 230 ± 11, respectively. The repeatability determined for each method was 19, 37, 28, 32, 7, 11, and 5%, respectively. Only method 5 (Leja slide) and the control (hemacytometer) methods provided results that were within the desired 10% repeatability. In addition, the sperm concentrations determined using the CASA methods were different from the control (P<0.05), and no slide preparation accurately determined sperm concentration (P <0.05). Precision among methods was not different (P >0.05) except for Method 4 (hanging drop), which was less precise than the other methods. Furthermore, the accuracy of the sperm concentrations was lower at the lowest sperm concentration. The results from the two CASA machines were not different from each other (P >0.05). Therefore, regardless of sperm preparation method, CASA does not accurately determine sperm concentration in canine semen. However, repeatability and precision of CASA-derived concentrations are maximized using fixed-chambered slides.

Keywords: Canine sperm concentration, computer-aided semen analysis, CASA slide preparation