The effects of method and operator experience on the accuracy of canine sperm counts
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Accurately measuring the concentration of spermatozoa in canine semen is of paramount importance as more veterinary practices offer semen evaluation, shipping and cryopreservation. Challenges commonly encountered when determining sperm concentration include cost of equipment, limited time, and lack of properly trained staff members to perform the procedure. The perfect quantification method for clinical use would be accurate, cost-effective, efficient and easy to perform. We hypothesized that the experience level of the operator would significantly impact the accuracy of the commonly used methods for measuring sperm concentration.

The improved Neubauer hemocytometer (HEMO) has long been considered the gold standard for the determination of sperm concentration in semen samples. While this is a cost-effective option, the process is tedious, time-consuming and presumably requires an experienced operator. This study compared the results obtained with the HEMO to those obtained with three commercially available methods, each representing a different cost and method of quantification. The commercially available methods used were the 591B Densimeter (DENS; Animal Reproduction Systems, Chino, CA), NucleoCounter® SP-100 (NC; Chemometec, Allerod, Denmark), and SpermVision SAR® computer assisted sperm analysis system (CASA; Minitube of America, Inc., Verona, WI). One hundred semen samples were obtained from a total of 18 healthy dogs of varying breeds over a three month period. Each sample was analyzed in duplicate by an experienced and inexperienced operator, using each of the four methods. The inexperienced operator was trained for a total of two hours among all methods. During this time, hands-on instruction was provided and two practice samples were counted for each method which were not included in the analyzed data. Proper procedure for each method was also posted in the laboratory so that the inexperienced operator could quickly refer to the protocols at any time, but no further hands-on instruction was provided.

Using a linear mixed-effects model, all three methods (DENS, NC, CASA) generated significantly biased estimates of concentration compared to HEMO ($F_{3,697}=86.05884; p<0.0001$). However, results indicated that the experience level of the operator did not influence the accuracy of any of the methods, as neither the main effect for experience ($F_{1,693}=0.15; p=0.70$), nor the interaction between experience and method ($F_{3,693}=2.19; p=0.09$), were statistically significant. Similarly, neither the interaction between sample number (i.e. experience gained over time) and experience, nor the interaction between sample number, experience, and method were significant ($F_{1,686}=0.48184, p=0.4878$; and $F_{3,686}=0.36027, p=0.7817$, respectively) indicating that the effects of experience on accuracy of methods were not influenced by the number of samples. Thus, experience level of the operator did not have a significant impact on accuracy for any of the methods tested.

**Keywords:** Spermatozoa, canine, concentration, accuracy, hemocytometer