ABSTRACTS

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Computer-assisted sperm analysis in dogs and cats: an update after 20 years of use

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Computer assisted sperm analysis (CASA) was originally described by Dott and Foster more than 30 years ago (1) and has gained increasingly more interest in veterinary medicine during the last decades. In dogs and cats, CASA was first described by Günzel-Apel et al. (2) and Stachecki et al. (3), respectively. Subsequently, numerous commercial CASA systems were validated for use in dogs and cats both for research purposes and various clinical applications. CASA systems offer an accurate, rapid and simultaneous assessment of different semen parameters such as concentration, total and progressive motility, slow, medium and rapid moving spermatozoa, linearity of sperm movement, the beat cross frequency, the amplitude of the lateral head displacement and different velocity parameters (4,5). Several studies described high correlations between the computer-calculated motility, progressive motility and concentration, and the conventional light microscopic evaluation (4,5). Additionally, these computerized measuring devices proved to be valuable for the detection of subtle changes in sperm motion which cannot be identified by conventional semen analysis. Moreover, high numbers of spermatozoa can be analyzed individually in a short period of time which make these systems practical for daily clinical use. The capability of detecting slight alterations in sperm movement was shown to be very useful for validating new sperm diluters, improving cooling and cryopreservation procedures and assessing the effect of drugs on sperm quality in dogs. Moreover, these systems can detect hyperactivation of spermatozoa, which is necessary for successful penetration of the zona pellucida. Furthermore, information obtained by CASA systems could be critically important when monitoring e.g. the effect of environmental or occupational stress on spermatozoa.

The main problems when using these computerized measuring devices are the relatively high investment costs, the need for standardization and validation of the system before any practical use is possible. The choice of internal image settings (e.g. minimum cell size, frame rate, analysis time) which is important to identify and reconstruct the trajectory of the different spermatozoa accurately, clearly influences the results obtained. In dogs, significant alterations of the motility characteristics measured by CASA systems have been described due to the dilution of the semen sample, the diluent used, the analysis temperature and the counting chamber (4,5). Once these systems have been standardized, a high degree of repeatability can be achieved with inter- and intra-assay coefficients of variation of less than 12% for most parameters. As a consequence, the computer parameters selected by the user, the software used and the microscopy conditions might lead to a new source of subjectivity among laboratories. However, due to the lack of uniformity among users and due to the use of different instruments, a definition of standard accepted values for motility and sperm velocity is difficult to determine in dogs. Standardization of the technical settings however could be important in order to compare results between laboratories and veterinary centers which may particularly be of importance in view of the increasing international exchange of cooled and frozen dog semen. Although several authors (4,5) provided large datasets of CASA measurements obtained from proven fertile dogs which could serve as reference values, it still needs to be determined which sperm movement characteristics are of clinical value for the prediction of in vivo fertility in dogs. Subtle changes in sperm motility characteristics and velocity patterns have been correlated with fertilizing ability in vitro and in vivo in several species, including rat, bull, stallion and dog. In human, the amplitude of the lateral head displacement was shown to affect the outcome of IVF. In one study including 111 dogs, the body weight of the dog was significantly correlated with the total sperm output and negatively correlated with the curvilinear velocity, and significant differences were detected between fertile and sub-fertile dogs for most of the evaluated sperm quality parameters assessed by the Hamilton-Thorne analyzer (HTR) (6).

Automated assessment of sperm morphometric dimensions and morphology has also been described in dogs by implementing e.g. the Metrix Oval Head Morphology software in the HTR system providing very detailed information on sperm head dimensions (length, width, area, roundness, perimeter), tail length and tail abnormalities (bent, coiled, absent). Based on these studies, dog sperm heads appeared to be smaller than bull, ram, goat and rabbit sperm heads but, interestingly, were larger than e.g. horse sperm heads. However, high variations in the sperm morphometric dimensions were found among individual dogs. Significantly lower morphometric dimensions of the canine sperm head were described after cryopreservation. Furthermore, the magnification level of the objective of the microscope, the sperm concentration and the staining method influence most of the morphometric dimensions obtained. High correlations were established for the percentage of normal spermatozoa assessed by light microscopic evaluation and by the HTR-Metrix. However, most of these automated systems for measuring sperm morphometric dimensions are time-consuming and remain to some extent inaccurate.
In contrast with dogs, CASA is rather poorly documented in felids (3,7). Most studies describe the characteristics of epididymal sperm which is frequently used for in vitro fertilization in cats (3,7). Recently reference values for cat epididymal sperm samples obtained after gradient density centrifugation were described which can facilitate comparisons of sperm quality and in vitro fertilization among laboratories. Improvement of biotechnical methods that result in fast and precise semen quality assessment is however required to further optimize assisted reproductive techniques in domestic cats and endangered wild felids.