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Sperm chromatin evaluation of raw dog semen using toluidine blue

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In recent years the reproduction of pets has reached an important development throughout the world. The main reasons are an increase in breeding purebred dogs for commercial purposes and use of the domestic dog as a model of wild canids in danger of extinction. This generates the need to develop new semen assessment techniques. Due to various reports that correlate the degree of DNA damage to various different fertility indices, the latter years has seen a growing interest to include DNA assays in routine semen evaluations. Toluidine Blue (TB) stain is a simple technique that has been used in different species to assess the degree of sperm chromatin condensation1,2,3.

The objectives of this study were to set up a technique to evaluate chromatin condensation in canine sperm and determine if it is possible to use dithiothreitol (DTT) as a positive control of the stain. A total of 21 ejaculates from 7 healthy canine males of different breeds, between 2 to 5 years old, were obtained by applying manual massage to the caudal portion of the bulbus glandis, collecting the first and second fractions of the ejaculate. The following routine semen characteristics were evaluated: volume (V), progressive motility (PM), concentration (C), membrane function (HOS) and normal sperm morphology (NM). The TB stain was used to assess the degree of chromatin condensation. Two staining times for the samples (15 and 30 minutes) and three staining times for the incubation with 1% DTT (2, 5 and 30 minutes) were also tested. Eight smears were made from each ejaculate: two of the sample and six of the sample incubated with DTT. Once dried, the smears were fixed with ethanol 96° for 2 minutes and finally stained with a working solution of 0.25% TB for both 15 and 30 minutes. A paired T test was used to compare the two staining times and descriptive statistics were performed on the samples incubated with DTT. Values for the routine semen characteristics were (mean ± SD): V: 1.5 ± 0.4 ml; PM: 79.4 ± 7.4 %; C: 344.1 ± 105.7 x10⁶ sperm/ml; HOS: 94.2 ± 1.3% and NM: 85.4 ± 3.6 %. The TB patterns observed were: light blue (negative, normal chromatin condensation), light violet (intermediate, some degree of decondensation) and dark blue-violet (positive, high degree of decondensation). No significant differences (p > 0.05) were observed between the males nor between the two staining times, for any of the TB patterns (negative: 95.1 ± 3.5% vs. 94.7 ± 3.2%; intermediate: 4.1 ± 2.8% vs. 4.6 ± 2.8% and positive: 0.8 ± 1.1% vs. 0.7 ± 0.8% for 15 and 30 minutes of staining, respectively). At all times of incubation with DTT (2, 5 and 30 minutes) 100% TB positive sperm were observed. This is the first report of the use of TB to evaluate canine sperm chromatin condensation. With regard to the two staining times assayed in this study, as no significant differences were observed either in the samples or in the controls, 15 minutes would be the staining time of choice as it shortens the duration of the assay. It was also verified that incubation with DTT can be used as a positive control of the TB stain in the canine species.