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A retrospective study on the relationships between semen quality, dogs’ ageing and fertility.
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Owners request semen evaluations of their dogs, however only a few Authors attempted to relate semen characteristics to age or fertility\textsuperscript{(1, 2)}. The aims of this study were to evaluate the effect of the dogs’ age on seminal characteristics and to relate main seminal characteristics with fresh AI results. Overall, 251 semen collections were performed from 145 dogs of 51 different breeds presented between 2000 and 2015 at the University of Pisa Veterinary Teaching Hospital. Semen, mainly the second fraction, was collected by manual stimulation, and was evaluated for volume by graded tubes, sperm concentration by Thoma counting chamber, and subjective motility under a contrast-phase microscope equipped with a warmed stage. Sperm morphology was evaluated since 2006 on smears stained with Spermac Stain® (Minitübe, Germany). Age groups (young: 0-24 months, adult: 25-84 months, old: >84 months) were compared by the Kruskal-Wallis Test, and post-hoc by Mann Withney test, used also to evaluate the differences between the ejaculates resulting in successful or unsuccessful AIs. Values are shown as medians and IQR. Differences are considered statistically significant when P<0.05. Median age of the dogs at collection was 63 months (IQR 59). Overall, samples containing spermatozoa were 226 (90.0%). Azoospermic samples were arising from complete (n=13, 9 dogs, 6.2%), or incomplete ejaculations (n=12, 8 dogs, 5.5%), based on seminal ALP concentration and/or ancillary examinations. Age did not influence significantly volume, sperm number or motility. The proportion of normal spermatozoa was higher in young animals (68.6% IQR 32.1) than in old ones (44.0% IQR 43.7; P<0.05). Midpiece defects were present in a significantly higher proportion of spermatozoa in old dogs (31.5% IQR 41.2), compared to young ones (12.5% IQR 20.2, P<0.01), and there were significantly more proximal droplets in the old animals (3% IQR 51.2; 44.4% of the old dogs had >10% sperm cells with proximal droplets) than in the other dogs (≤1%; P<0.01). A tendency for a higher proportion of normal spermatozoa (66.1%) and a lower proportion of midpiece defects (15.5%) was seen in adult compared to old dogs (P<0.06). Of the 65 bitches inseminated with fresh semen, 47 (72.3%) whelped. Median sperm number, motility and proportion of morphologically normal spermatozoa of the ejaculates used in AIs resulting in a pregnancy were 560.0x10\textsuperscript{6}, 85% and 72%, respectively, significantly higher than in the unsuccessful AIs, where they were 312.0x10\textsuperscript{6}, 75% and 25%. Age did not affect AI results. These results show how with age the proportion of normal spermatozoa decreases and midpiece defects, especially proximal droplets, increase. It has been reported how this sperm defect can be associated with infertility in the dog\textsuperscript{(3)}. In mature bulls proximal droplets are indicative of a degenerative process of the seminiferous epithelium. It can thus be hypothesized that the increase of this abnormality seen with age in dogs, may derive from a senile testicular degeneration. The greatest difference between samples used for AI resulting in a pregnancy or not was for sperm morphology. In conclusion, this retrospective study underlines the importance of sperm morphology, and how this deteriorate with ageing.