ABSTRACTS

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Cold storage of canine semen: the in vitro effect of different concentrations of a combination of antibiotics on bacterial growth

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OBJECTIVES AND METHODS: Mycoplasma isolation rates have been proven to be significantly higher in semen samples from infertile than from fertile dogs (1). During cooled-storage of stallion semen, gentamicin is more effective in the reduction of bacterial growth than other antibiotics (2). However, gentamicin concentration has to be optimized to prevent detrimental effects on spermatozoal function (3). In bulls, a combination of gentamicin, tylosin and lincomycin was highly effective for the control of mycoplasmas (Mycoplasma spp., Ureaplasma spp.). (4). The aim of the present study was to examine the effect of different concentrations of antibiotics on growth of bacteria including mycoplasmas during cooled-storage of extended canine semen. Semen was collected by digital manipulation from 11 stud dogs (mean age ± SD: 3.6 ± 2.6 years) of different breeds. Prior to division of each ejaculate into four aliquots, a sample for bacterial culture was taken. Growth of micro-organisms was quantitatively determined as low (4-100 colony forming units (CFU/µl)), moderate (101-500 CFU/µl) or high (>500 CFU/µl). Quality of the sperm-rich semen fraction was assessed with regard to volume, concentration, pH, number of PMN and percentage of morphological aberrations. Motility and viability were assessed via computer-assisted sperm analysis (CASA; Spermvision, Minitüb, Tiefenbach, G). Each semen aliquot was diluted 1:2 with one of the following extenders (Tris-citric acid-fructose-egg yolk; 5), containing either streptomycin and bencylpenicillin (A; g/l) or increasing concentrations of tylosin, gentamycin, lincomycin and spectinomycin (B,C,D; g/l): (A) [1 and 0.6 g/l] (B) [0.05, 0.25, 0.15 and 0.3 g/l] (C) [0.1, 0.5, 0.3 and 0.6 g/l] (D) [0.2, 1.0, 0.6 and 1.2 g/l]. Each diluted sample was immediately assessed for motility parameters and viability (d0) as well as after 24 (d1), 48 (d2) and 72 (d3) h of cold storage (+5°C). Determination of morphological aberrations and bacterial culture of each aliquot were performed after 24 and 72 h.

RESULTS: Average values of motility, progressively motile sperm, viability and morphological aberrations did not differ between groups (p > 0.05). In raw semen, in eight of 11 samples (72,7%) high grade growth and in one sample (9%) moderate growth of mycoplasmas occurred while in two samples (18.2%) no mycoplasmas were detected. On d3, in group A, there were still 7 samples (87.5%) with high grade growth of mycoplasmas; in B, in six samples (75%) mycoplasma growth was reduced to moderate, and in C, in four samples (50%) a decrease from high grade to moderate and in two samples (25%) to low grade was found. Two samples (25%) remained with high grade growth. With the D extender, no sample with high grade growth of mycoplasmas was detected after 72 h; in one sample (12.5%) growth was reduced from high grade to moderate and in seven samples (87.5%) to low grade.

CONCLUSION: We conclude that dilution of canine semen with a Tris-citric acid-fructose-egg yolk containing 0.2, 1.0, 0.6 and 1.2 g/l of tylosin, gentamycin, lincomycin and spectinomycin is not detrimental to semen during cooled-storage for 72 h. However, it significantly reduced the growth of mycoplasmas compared to the extender containing streptomycin and bencylpenicillin.