Effect of diluent composition and incubation temperature on survival time of fresh spermatozoa of fallow deer (*Dama dama*) and red deer (*Cervus elaphus*)

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The effect of various parameters on the survival time of spermatozoa of fresh fallow deer (Dama dama) and red deer (Cervus elaphus) for use in artificial insemination programme was investigated. Semen samples were collected during the breeding season from fallow deer bucks (n=4) and red deer stags (n=3) by electroejaculation. Following collection, the semen samples were assessed for quality and then diluted to a concentration of 100x106 or 20x106 spermatozoa/ml. In Experiment 1 the semen was diluted in Caprogen (Shannon, 1965) or a synthetic diluent (RSD-1: Upreti et al., 1991) and stored at 37°C or 20°C. In Experiment 2, the semen was diluted in Caprogen and stored at 37°C, 20°C or 4°C. In both experiments subsamples of semen stored at 20°C (Experiment 1) or 20°C and 4°C (Experiment 2) were transferred to 37°C 48 hours after start of the experiments. The proportion of live spermatozoa was determined at 12-hourly intervals and acrosome status was determined using the Lenz stain and electron microscopy on spermatozoa in fresh ejaculates and in diluted semen at 48 hours after start of each experiment.

The average volume and concentration of semen (s.e.) for fallow (n=8) and red (n=6) deer were 1.3 (0.2) ml and 1.3 (0.4) ml and 2281 (283) (x10⁶)/ml and 1138 (404) (x10⁶)/ml respectively. The percentage of acrosome intact spermatozoa

(s.e.) in the ejaculates of fallow and red deer were 98.5 (0.5)% and 98.0 (0.6)% respectively. In Experiment 1, survival time of spermatozoa was longer following dilution with Caprogen than RSD-1 and there was a significant prolongation of survival time with reduced incubation temperature (P<0.01). Survival time (s.e.) following incubation of 20x106/ml spermatozoa at 20°C in Caprogen vs RSD-1 was 237.7 (9.6)h vs 118.7 (1.1)h for fallow deer and 228.3 (8.9)h vs 111.7 (4.4)h for red deer. In Experiment 2, survival time of spermatozoa (s.e.) was significantly longer following storage at 4°C than 20°C (P<0.01; fallow deer: 585.7 (11.4)h vs 220.5 (1.5)h, red deer: 292.9 (4.8)h vs 203.2 (3.7)h; data are for spermatozoa diluted at 20x106/ml). Following transfer to 37°C, spermatozoa of both species remained alive for approximately 48h with no differences between initial storage at 20°C or 4°C. The percentage of acrosome intact spermatozoa was lower (P<0.01) following incubation at 4°C than 20°C in both species (fallow deer: 76.8 (4.2)% and 91.8 (0.8)%; red deer: 51.6 (3.2)% and 85.0 (1.9)%.

These data present Caprogen as a diluent suitable for preservation of fresh spermatozoa at ambient temperature. This may offer a practical option for national artificial insemination programmes in cervid species.

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