CRYOBANKING OF SPERMATOZOA FROM A BARKING DEER (*MUNTIACUS UNTIACUS*): AN INNITIATIVE TO CONSERVE GERMPLASM OF WILDLIFE IN SRI LANKA

G.D.R.K.PERERA[1], M.G.D.S.B.MEEGAHAKOTUWA[1], M.L.A.N.R.DEEPANI[1], L.N.A.DE SILVA[1], P.G.A.PUSHPAKUMARA[1], W.A.KING[2] AND B.ALEXANDER[1]

Animal Embryo Biotechnology Laboratory, Department of Farm Animal Production and Health, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya.
[2] Department of Biomedical Science, University of Guelph, Ontario, Canada.

Cryobanking of spermatozoa plays an important role in conservation of genetic resources of animal species. There have been no attempts made to freeze germplasm of wild life species in Sri Lanka. Therefore, the aim of this study was to develop a technique to collect and preserve spermatozoa of wild animal species that are threatened to extinct in Sri Lanka.

Testes of a sexually mature barking deer were collected immediately after death which was brought to the Veterinary Teaching Hospital, University of Peradeniya. A portion of the body and tail of the epididymis and proximal part of the vas deference were removed and placed in 5 ml cattle semen extender (extender A, without glycerol) at 37^0 C and sliced into small pieces. The sliced sections were kept in the extender for 10 minutes at 37^0 C, for the spermatozoa to ooze out from the tubules.

After evaluation of motility and morphology of the sperms, the slurry was filtered through sterilized gauze and the clean sample was collected into a conical tube. Then spermatozoa in extender A and the B portion of the extender (containing glycerol) were kept at 4^0 C in the refrigerator for 2 h. The portion B of the extender was added drop wise into the extender A with spermatozoa for a dilution of 1:1 until a final sperm concentration of 80×10^6 sperms/ml was achieved. Extended spermatozoa were loaded into Mini-straws, using a mouth apparatus and placed at 4^0 C for 4 h. The straws were placed in a liquid nitrogen vapour for 15 minutes, loaded into cold goblets and stored at -196^0C in liquid nitrogen.

After thawing at 35^0 C for 10minutes, banked sperms were evaluated. The progressive motility of the spermatozoa was 90 % and 80 % at day 01 and 75 days post cryobanking, respectively. At the detail evaluation of spermatozoa, 1.5 % of abnormal sperm heads, 6% of proximal cytoplasmic droplets, 2 % of distal cytoplasmic droplets, 0.5 % of tailless heads, 3% of singly bent tails, 1 % of doubly bent tails, 2 % of coiled tails and 0.5 % of structural abnormalities of midpiece were found. After thawing, live: dead ratio was 83:17 on day 75 post cryobanking. Twenty four straws were prepared and cryobanked in liquid nitrogen. The results revealed that spermatozoa from barking deer can be frozen successfully in liquid nitrogen. This study highlights the possibility of cryobanking of germplasm of wildlife which are threatened to extinct as a conservation strategy in Sri Lanka.

Funded by Council of Agricultural Research Policy, (12/660/498), Sri Lanka and the Department of Biomedical Science, University of Guelph, Ontario, Canada.