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Collection and cryopreservation of semen of the Hokkaido brown bear
(*Ursus arctos yesoensis*)

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The Hokkaido brown bear (*Ursus arctos yesoensis*) is found only in Hokkaido Island, Japan, and recently habitats of this bear have been fragmented and shrunken. It has been reported that their genetic diversity is generally low. Obtaining baseline data necessary for the application of artificial breeding techniques to this bear is important and effective for conservation of the genetic resource of this bear.

The objectives of the present study were 1) to determine the effectiveness of electroejaculation for collecting bear spermatozoa, 2) to document seminal traits of captive Hokkaido brown bears, 3) to clarify the optimal condition for freezing of the semen of the bear, and 4) to investigate the seasonal effects on seminal traits and endocrinological status.

In the first chapter, semen collection was conducted using electroejaculation in captive anesthetized bears, and spermic ejaculates were recovered. Mean semen volume was 2.7 ml, and mean pH of semen was 7.2. Mean values of sperm concentration, sperm motility, the percentage of live spermatozoa and sperm abnormality rate were 320.9×10^6 cells/ml, 78.4%, 85.3% and 20.3%, respectively. Considerable variations in seminal traits were observed between trials and individuals. Sometimes urine contaminated into semen, and low pH and sperm motility were observed in contaminated semen.

In the second chapter, semen of the bear

was frozen with the methods used for the semen of domestic animals and the other wildlife. The traits of post-thaw semen were compared among different freezing conditions, such as semen extender, cooling rate, final glycerol concentration, and equilibration time. In this study, the best conditions for freezing of bear semen were that dilution with Tris-buffered egg yolk extender, 4.7% of final glycerol concentration, 80 min of equilibration time, and slow cooling rate (7.5°C/min from 5°C to -7°C). Under those conditions, the mean sperm motility and the mean percentage of live spermatozoa were 36.0% and 65.0%, respectively.

In the third chapter, annual changes in serum testosterone concentration, serum FSH concentration, and seminal traits were investigated. Serum testosterone concentration was high in May and June, and low from July to April. The increase in serum testosterone concentration was followed by the increases of sperm concentration. The pattern of the annual change of serum FSH concentration was similar to that in testis volume.

The present study is the first report on successful semen collection and cryopreservation in the Hokkaido brown bear. In addition, annual changes of seminal traits, serum testosterone concentration and serum FSH concentration were revealed in the present study.