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cient for follicle aspiration.

At the end, the OPU from live cows was performed using follicle aspiration system with long and short single-lumen needles and a long double-lumen needle attached to two kinds of hand-made probe-carrier. The system with a long single-lumen needle significantly decreased the COC recovery rate and the proportion of normal COCs with several cumulus layers. The COC recovery rate in a long double-lumen needle

system had the highest percentage, but was not statistically different from that in the short single-lumen needle system. These results indicate that follicular aspiration systems with a long double-lumen needle and a short single-lumen needle are appropriate for the OPU in cattle. As for handling and cost of needle, a short single-lumen needle is better than a long double-lumen needle.

Establishment of fecal progesterone and testosterone assays  
by enzyme immunoassay technique and their application to differentiate  
sexes and sexual maturity in Hokkaido brown bear

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Hokkaido brown bear (*Ursus arctos yesoensis*) is uniquely found in the Hokkaido island of Japan. It is essential to know the number of individuals having reproductive capability to estimate a trend of population. Measurement of non-invasive fecal steroid hormones could be applied to evaluate their reproductive status. This study was conducted to establish assay systems for fecal progesterone and testosterone by enzyme immunoassay (EIA), and to apply the EIA hormone assay systems to differentiate sexes and sexual maturity in Hokkaido brown bears.

Double antibody EIAs for progesterone and testosterone were established. The detectable ranges were  $1.95 \times 10^{-2} \sim 1.25$  ng/ml for progesterone, and  $4.88 \times 10^{-3} \sim 1.56 \times 10^{-1}$  ng/ml for testosterone. Intra- and inter-assay coefficients of variation for the progesterone and testosterone assays were 10.9 and 16.4%, and 8.1 and 11.6%, respectively.

The conditions of extraction using the feces obtained from captive bears were investigated. Moreover, the coefficients of correlation between plasma and fecal concentrations collected from immobilized bears were determined. The recovery rates of added progesterone or testosterone to the wet feces were consistent through the effective ranges of the assays. The mean recovery rates were  $29.8 \pm 9.3\%$  for progesterone and  $58.8 \pm 13.9\%$  for testosterone. There was a significant correlation between plasma and fecal testosterone concentrations ( $r=0.75$ ,  $p<0.001$ ); however, no correlation was found between plasma and fecal progesterone concentrations ( $r=0.06$ ,  $p=0.73$ ).

Feces of mature and immature of both sexes were collected from captive bears once a month from December to July. From December to February ( $p<0.05$ ), especially in December ( $p<0.01$ ), mature female had significantly higher fecal progesterone concentration than the other

groups. Except March and April, mature male had higher fecal testosterone concentration ( $p < 0.05$ ), this was especially apparent in May ( $p < 0.01$ ). These results suggest that the EIAs are useful for measurement of fecal progesterone and

testosterone of Hokkaido brown bears, and that sex and sexual maturity of individuals could be determined by the measurement of fecal progesterone and testosterone concentrations.

Development of serological test procedure to specify the endemic foci of tick-borne encephalitis and the seroepidemiological survey in Hokkaido.

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In October 1993, a human case of encephalitis was diagnosed as tick-borne encephalitis (TBE) in Kamiiso, Hokkaido. As transmission cycle of TBE virus was proven to exist in this area, TBE virus may be endemic not only at the area where a patient was found but also in other parts of Japan. To specify the location of TBE endemic foci, a seroepidemiological survey was performed among horses and dogs in Hokkaido and then wild rodents in the antibody-positive areas using neutralizing (NT) test. Furthermore, seroepidemiological survey was extended to human and cattle cases which were diagnosed as encephalitis. A possible existence of TBE virus in Hokkaido for many years was discussed by retrospective study using cattle sera collected in 1978. Results are summarized as follows:

1. Horse sera collected in 1992 and 1998 were examined by NT test. TBE specific antibody was detected in 8 (Shiribeshi, Hiyama, Hidaka, Nemuro and Oshima district) of 1,695 horse sera. Out of 193 dog sera, 18 sera collected in

Shiribeshi and Oshima district had TBE specific antibodies.

2. Rodents were captured in the 6 positive areas revealed by the survey of horse and dog. TBE specific antibodies were detected in rodent sera collected from 5 areas including that where the TBE patient was found and neighboring towns. The results indicate that TBE virus has been endemic in the southern part of Hokkaido.

3. From the above results, TBE positive areas can be confirmed by testing horse or dog sera first and rodent sera later.

4. Although sera were collected from both human and cattle cases diagnosed as encephalitis in Hokkaido and Honshu, none of the sera were positive for TBE antibody.

5. No TBE positive sera were found in wild animals in Honshu.

6. Cattle sera collected in 1978 were tested, and one sample from Abashiri district was positive for TBE antibody. This result suggests that TBE virus may have been endemic in Hokkaido since at least 1978.