Some of the oocytes were treated with A23187 for 10 min after ICSI. Treatment with A23187 activated more than 90% of oocytes regardless of ICSI procedure. However, treatment of the injected oocytes with A23187 had no effect on the induction of sperm head decondensation in both the conventional ICSI and the piezo ICSI groups. The proportion of activated oocytes with decondensed sperm head was higher in the piezo ICSI groups (around 70%) than in the conventional ICSI groups (around 5%) regardless of A23187 treatment. In experiment 2, effects of freezing and thawing of the spermatozoa to lose acrosomal cap were examined. The proportions of the spermatozoa without acrosomal cap and the live spermatozoa were determined in non-treated spermatozoa, which were motile spermatozoa selected by Percoll centrifugation, and frozen-thawed spermatozoa used in experiment 1. Three percent of the non-treated spermatozoa and 67% of the frozen-thawed spermatozoa were without acrosomal cap. When the two kinds of bovine spermatozoa were injected into the *in vitro* matured bovine oocytes using piezo device, the percentages of oocytes with sperm head decondensation (72 vs. 71%) were not significantly different between the two sperm treatment groups.

The data concluded that both procedures for induction of oocyte activation and treatment for acrosomal cap loss are not essential for sperm head decondensation following bovine ICSI. Further experiments are needed to elucidate the effects of these treatments on pronuclear formation and subsequent development of bovine zygotes produced by ICSI.

Effects of follicular aspiration system on the results of transvaginal ultrasound-guided ovum pick-up in cattle

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Recently, the transvaginal ultrasound-guided ovum pick-up (OPU) has been developed to collect oocytes from live cows for *in vitro* production of bovine embryos. Although various OPU systems have been developed and used, the systems can be improved in terms of ways; the recovery rate of cumulus-oocyte complex (COC), ease of handling and cost. This study was carried out to develop an OPU system in cattle, where twisting and type of aspiration needles, and tubing system were emphasized.

First, the effect of twisting the needle during follicular aspiration on the recovery rate of COCs was investigated using ovaries from slaughterhouse, by a long single-lumen needle. The results revealed that needle twisting increased the recovery rate of oocyte with cumulus cells.

Secondly, the effects of vacuum pressure on the COC recovery rate were examined using four OPU systems assembled with different type of needles (long or short single-lumen, or long double-lumen) and tubings with or without junctions. As the results, the most suitable vacuum pressure (flow rate) observed for follicle aspiration was 50 or 75 mmHg (7.5–15.0 ml/min) in all OPU systems. It was thus, observed that tubing with junctions reduces the COC recovery rate, double-lumen needle was suitable and effi-

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 1.95×10^{-2}~1.25 ng/ml for progesterone, and 4.88×10^{-3}~1.56×10^{-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±9.3% for progesterone and 58.8±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