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## Pathological Analysis of Cerebellar Lesions In Human IL-2 transgenic mice

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Human IL-2 (hIL-2) transgenic mice (hIL-2 mice) show ataxic gait starting at 12 days of age. The ataxia is known to be associated with severe infiltration and tissue destruction in the cerebellum, but the pathogenesis of the cerebellar lesions is incompletely understood. The present study was carried out to characterize inflammatory processes of cerebellar lesions in hIL-2 mice.

Histopathologically, the cerebellar lesion was first detected at 4 days of age. At the early stage of the lesion, lymphocytes, neutrophils and macrophages accumulated in the subarachnoid cavity. Large granular lymphocytes (LGL), neutrophils and macrophages infiltrated into the parenchyma at older ages. The increase in the number of infiltrated cells correlated with the severity of parenchymal destruction via apoptosis

and demyelination. The cerebellum became extremely small by postnatal day 18.

Reverse transcriptase-PCR was used to determine cytokine mRNAs in the cerebellum. The expression of hIL-2 mRNA was detected in hIL-2 mice between embryonic day 16 and postnatal day 18. Interferon (IFN)- $\gamma$  mRNA expression in hIL-2 mice was stronger than that in control mice.

These results suggest that LGLs, macrophages and neutrophils are responsible for the destruction of cerebellar parenchyma in hIL-2 mice. Expression of hIL-2 and IFN- $\gamma$  is likely to promote local proliferation and/or activation of inflammatory cells, rather than directly causing tissue injury. Apoptosis of intrinsic cerebellar cells may play a role in the development of the cerebellar lesion.

## Intracytoplasmic sperm injection in the cattle: effects of oocyte activation and sperm treatment on sperm head decondensation

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In the present study, efficiency of oocyte activation and sperm treatment on sperm head decondensation in association with bovine intracytoplasmic sperm injection (ICSI) were investigated. In experiment 1, effects of additional treatment of the injected oocytes with calcium ionophore (A23187) on sperm head decondensa-

tion were examined at 4-6 hr of culture after ICSI. Frozen-thawed bovine spermatozoa, which had been incubated with heparin and frozen without cryoprotectant, were injected into bovine oocytes matured *in vitro* using a manually-driven fine glass pipette (conventional ICSI) or an electrically-driven glass pipette (piezo ICSI).

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 slaughter-

house, 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-